ADSORPTION OF POLYLYSINES AT SOLID-LIQUID INTERFACES

ISBN=211301-03

Promotor: dr. J. Lyklema, hoogleraar in de fysische en kolloïdchemie

STELLINGEN

1. Bumbullis *et al.* en Wolfes en Schügerl noemen de bulk-viscositeit die zij berekenen m.b.v. gegevens verkregen uit transversale capillaire golf- en oppervlaktespanningsmetingen aan viscoelastische oppervlakken ten onrechte de oppervlakte-viscositeit.

   Bumbullis, W., Kalischewski, K en Schügerl, K. (1981). Foam behaviour of biological media. VII. Surface viscosity and viscoelasticity. Eur. J. Appl. Microbiol. Biotechnol. 11, 110-115.
   Wolfes, H. en Schügerl, K. (1983). Foam behavior of biological media. VIII. Surface properties. Eur. J. Appl. Microbiol. Biotechnol. 17, 371-375.

2. De Voeght en Joos missen een essentieel punt in het gedrag van de capillaire golven op het vloeistofoppervlak van natriumlaurylsulfaat/1-tetradecanol oplossingen door niet op te merken dat in dit geval de modulatie van de amplitude van de golven met de afstand wezenlijk verschilt met de amplitudemodulatie die bij water t.g.v. reflecties optreedt.

   Voeght, F. de en Joos, P. (1983). Waves at the air/liquid interface of a surfactant solution with a high surface shear viscosity. J. Colloid Interface Sci. 95, 142-147.

3. Bij een lage en constante geadsorbeerde hoeveelheid vindt t.g.v. de ladingstegenstelling tussen adsorbaat en adsorbens in poly-L-lysine, geadsorbeerd aan polystyreen (latex), geen ladingsgeïnduceerde helix-kluwen overgang plaats. Bij hogere adsorpties is zo'n overgang echter wel mogelijk.

   Dit proefschrift, hoofdstuk 6.

4. De analyse van de Surface-Enhanced Raman Spectra (SERS) van piperidine van Sanchez *et al.* zou volledíger zijn geweest als zij er ook de invloed van de bezettingsgraad van het adsorbaat bij hadden betrokken.

   Sanchez, L.A., Birke, R.L. en Lombardi, J.R. (1984). Surface-Enhanced Raman scattering of piperidine. The effect of electrode potential on intensity. J. Phys. Chem. 88, 1762-1766.

5. Bij de presentatie van polyelectrolytadsorptiegegevens, waarbij de geadsorbeerde hoeveelheid is uitgedrukt als hoeveelheid massa, dient het duidelijk te zijn of het tegenion wel of niet hierin is inbegrepen.

6. Het onderscheid dat Lok *et al.* maken tussen reversibel en irreversibel geadsorbeerd runder plasma-albumine is niet zinvol omdat zij de aggregatietoestand van het eiwit niet hebben vastgesteld.

Lok, B.K., Cheng, Y-L. en Robertson, C.R. (1983). Protein adsorption on crosslinked polydimethylsiloxane using total internal reflection fluorescence. J. Colloid Interface Sci. 91, 104-116.

7. De grafieken van $Y'/\omega^{\frac{1}{2}}$ *versus* ω die Kvastek en Horvat gebruiken om de Warburg coëfficiënt uit te rekenen, geven alleen de door hen verwachte rechte te zien omdat ze het (wel gemeten) hoogfrequente deel ervan weglaten.

Kvastek, K. en Horvat, V. (1981). Kinetic study of the Ag/AgI electrode by complex impedance dispersion analysis. J. Electroanal. Chem. 130, 67-79.

8. Bij het onderzoek naar het gebied van afschuifsnelheden waarbij men pseudo-plastische vloeibare levensmiddelen in de mond beoordeelt op hun dikvloeibaarheid, gaan Cutler *et al.* ten onrechte voorbij aan het feit dat de meeste van deze vloeistoffen ook thixotroop zijn.

Cutler, A.N., Morris, E.R. en Taylor, L.J. (1983). Oral perception of viscosity in fluid foods and model systems. J. Text. Stud. 14, 377-395.

9. Bij het nagaan van het verband tussen de geadsorbeerde hoeveelheden polyacrylzuur en de wandlading van de adsorbentia hematiet en rutiel, houden Gebhardt en Fuerstenau ten onrechte geen rekening met een verschuiving van de wandlading die kan optreden t.g.v. de adsorptie van het polyelectrolyt.

Gebhardt, J.E. en Fuerstenau, D.W. (1983). Adsorption of polyacrylic acid at oxide/water interfaces. Colloids and Surfaces 7, 221-231.
Dit proefschrift, hoofdstuk 4 en 5.

10. De schatting die Buscall en Corner maken van de gemiddelde volumefractie polyacrylzuur aan het oppervlak van hun polystyreendeeltjes, op basis van o.a. protontitraties van geconcentreerde polyacrylzuuroplossingen, is inconsistent met een van de door hen gemaakte veronderstellingen.

Buscall, R. en Corner, T. (1982). Polyelectrolyte stabilised latices. Part 2: Characterisation and colloidal behaviour. Colloids and Surfaces 5, 333-351.
Dit proefschrift, hoofdstuk 6.

11. In tegenstelling tot de resultaten beschreven in dit proefschrift vinden Furusawa *et al.* geen noemenswaardige invloed van de zoutconcentratie op de adsorptie van poly-L-lysine aan polystyreen (latex) bij pH 4. Waarschijnlijk is dit het gevolg van de meetprocedure die ze toepassen.

Furusawa, K., Kanesaka, M. en Yamashita, S. (1984). Adsorption behavior of poly-L-lysine and its conformation at the latex-water interface. J. Colloid Interface Sci. 99, 341-348.
Dit proefschrift, hoofdstuk 4.

12. In theoretische publicaties waarin de resultaten van computerberekeningen wezenlijk zijn voor de theorie, dient de beschrijving van de bijbehorende programma's minstens gelijkwaardig te zijn aan die welke gangbaar is bij de paragraaf materialen en methoden in experimentele verhandelingen.

13. Een grotere integratie van beleid m.b.t. waterkwaliteit en waterkwantiteit dan nu in Nederland gebruikelijk is, kan leiden tot een grotere doelmatigheid van het waterbeheer.

14. De consumptie van zoutjes bij het televisiekijken kan toenemen door de aanwezigheid van ondertitels.

B.C. Bonekamp
Adsorption of polylysines at solid-liquid interfaces
Wageningen, 12 september 1984

B.C. Bonekamp

ADSORPTION OF POLYLYSINES AT SOLID-LIQUID INTERFACES

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
in het openbaar te verdedigen
op woensdag 12 september 1984
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

Voor mijn ouders

Voor Margo, Stijn en Bart

ABSTRACT

Bonekamp, B.C. (1984) Adsorption of polylysines at solid-liquid interfaces. Doctoral thesis, Agricultural University, Wageningen. 208 p, 80 figs, 5 tables. English and Dutch summaries

Adsorption properties of the polyelectrolytes poly-L-lysine (PL-L) and poly-DL-lysine (PL-DL) on hydrophobic (polystyrene latex, silver iodide) and hydrophilic (silica) negatively charged solid particles were studied.

Adsorbed amounts as a function of concentration, ionic strength, surface charge, PL chain length and chain charge density were determined. The adsorption of PL on negatively charged polystyrene latex and silica was also monitored conductometrically and potentiometrically. Further flocculation and coagulation measurements were performed. Information about the secondary structure of adsorbed PL-L was obtained from proton titrations of PL-L and PL-DL adsorbed at polystyrene particles.

The stereoregularity and secondary chain structure of PL does not influence the adsorbed amount. At low constant adsorbed amount, there is no coil to helix transition in adsorbed PL-L, because of the charge contrast and hydrophobic interactions between PL and the surface. Only when the adsorbed amount is high at each pH, a transition takes place and adsorbed PL-L can be partly helical.

At low pH and low ionic strength the adsorbed polyelectrolytes show a rather flat conformation and there is no pronounced effect of the hydrophobicity of the adsorbent. All negative surface groups form ion pairs with an $-NH_3^+$ group of PL, but the reverse is not the case. The adsorption of the basic polyaminoacids increases (i.e. loops and tails start to develop) if the pH is increased or if the ionic strength is raised. With the hydrophobic adsorbents the electrolyte effect persists up to very high concentrations. In the case of the hydrophilic silica no increase above 0.01 M salt was observed, because here hydrophobic interactions are absent. The ionic strength and pH dependence of the adsorption on hydrophobic substrates are in satisfactorily agreement with theoretical predictions.

Free descriptors: Adsorption, binding, solid-liquid interface, polycation-polyanion complex, polyelectrolyte, charged macromolecule, charged polyaminoacid, poly-L-lysine, poly-DL-lysine, PLL, PLDL, polystyrene latex, silica, conformation, conformational transition, helix-coil transition, secondary structure, proton titration, conductivity, flocculation, coagulation.

CONTENTS page

1 INTRODUCTION

1.1 GENERAL BACKGROUND

Interactions between polymers of biological or synthetic origin and interfaces are of importance for a wide variety of biological, medical and technological processes. For instance bound proteins and glycoproteins play important roles in natural membranes (see e.g. *Cantor & Schimmel, 1980*). Further, the flocculation of suspended bacterial cells by biological or synthetic water-soluble polyelectrolytes is of considerable importance in biotechnology and water purification (*Daniels, 1980*). Also the attachment of whole bacterial cells or viruses to solid-surfaces is an example of biopolymer-surface interaction and of relevance for microbiology, biochemistry, (bio)engineering and dentistry (*Daniels, 1980*). Still more examples will be given in the following chapters. Because of the importance of polypeptides including the proteins at biological and non-biological interfaces, I shall now focus on the adsorption properties of water soluble polypeptides only.

Polypeptides appear in nature at various levels of structural organization, from random coils up to ordered secondary, tertiary and quarternary structures*. In view of the structure-function relation of natural polypeptides the investigation of the three dimensional structure of polypeptides, proteins included, is of great importance. The conformation (i.e. three-dimensional structure) of a polypeptide is the result of the sum of all intra molecular interactions and interactions of the biopolymer with the environment. As the presence of an adsorbent implies a change of this environment it is reasonable to assume that the conformation of polypeptides can change upon adsorption.

Another important aspect concerning the adsorption of most natural polypeptides is, that these polypeptides contain aminoacid residues with ionizable side chains. Hence these polypeptides can be electrically charged and the electrostatic interaction with interfaces, which are also charged in most cases, may to a greater or lesser extent influence

---

* The primary structure of a polypeptide is defined as the sequence in which the amino acids are linked together. The secondary structure refers to the conformation the polypeptide backbone adopts (e.g. α-helix and β-structure). The tertiary structure refers to folding of sections of secondary structure in space. The quaternary structure refers to the arrangement of tertiary structures in space.

the adsorption properties. It should also be realized that adsorption from solution is a competitive process. When polypeptide molecules adsorb, solvent (water) molecules and possibly other components (e.g. ions) must be displaced. In general, the structure of the adsorbed polypeptide layer will be the net result of all intra- and intermolecular interactions including the interactions of the polypeptide with the solvent and the surface.

When a polypeptide molecule in solution behaves as a random coil, i.e. as a flexible polymer chain, the adsorption behaviour should resemble that of synthetic flexible polymers. Such polymers adsorb at almost any surface and the adsorption isotherm is usually of the High Affinity type. Even if the affinity of a polymer segment for the surface is small, the adsorption is still of a high affinity type, because the total adsorption energy is the sum of the binding energy of many segments. Not all segments of the adsorbed macromolecules need to be attached to the surface. Part of them can reside in loops and tails protuding into the solution. A series of adjacent segments in contact with the surface is called a train. The adsorbed layer consists then of trains, loops and tails. The interfacial properties are strongly influenced by the presence of such an adsorbed layer (see e.g. *Fleer and Lyklema (1983)*).

When there are considerable intrasegmental attractions in a polypeptide molecule, such as hydrophobic bonds, hydrogen bonds, ionic interactions etc., the train-loop-tail model is not suitable for the description of the adsorbate. This is the case for most proteins. As a consequence of the strong intra molecular attractions most proteins have a well-defined three dimensional structure, containing parts with different degrees of order ranging from random coils to α helix and β sheet structures. Because of the specific structures they adopt in solution, a general theory for the adsorption of proteins does not exist at present.

Another complicating factor with proteins as adsorbates is that they are usually ampholytic polyelectrolytes. The importance of electrostatic and hydrophobic interactions in determining the properties of adsorbed proteins has been emphasized by for instance *Norde and Lyklema (1978)*.

Over the past three decades synthetic polypeptides (polyaminoacids) have evolved as an interesting class of polymers in their own right, and as model proteins for conformational studies. Much work has been done on the properties of synthetic poly-α-aminoacids in the solid

state and in solution (*Fasman, 1967*). However relatively little attention has been directed to their interfacial properties especially at solid-liquid interfaces. Among these interfacial studies a minor part is devoted to the study of charged poly-α-aminoacids capable of forming secondary structures. This despite of the importance of charge interactions and structural alterations in adsorbed proteins (see e.g. *Norde, 1978*). Although the study of the adsorption of charged polyaminoacids gives no direct information about the behaviour of tertiary polypeptide structures at interfaces, the study of these compounds can provide insight in the behaviour of α helices and other secondary structures at surfaces and the role of charge interactions therein. Also the role of the aminoacid side chains in the surface interaction can be traced. It is therefore that this thesis is devoted to polylysine, a positively charged poly-α-aminoacid capable of forming secondary structure, adsorbed at solid-liquid interfaces. As at low pH polylysine is a highly charged polyelectrolyte, the adsorption properties will resemble then those of other synthetic polyelectrolytes and they can be compared with theoretical predictions of *van der Schee (1984)* for the adsorption of flexible polyelectrolytes.

1.2 AIMS OF THIS WORK

The aims of the present study are:

(i) To gain more insight into the behaviour of charged polymers at (oppositely) charged interfaces and to compare the results with theoretical predictions and with the homogeneous complex formation between polycations and polyanions in solution.

(ii) Establishing of the role of the substrate in determining the characteristics of the adsorbate.

(iii) Establishing of the secondary structure of adsorbed weak (cationic) acid polyelectrolytes at low chain charge density and the occurrence of conformational transitions in the adsorbed state.

1.3 SYSTEMS USED AND OUTLINE OF THIS STUDY

Most experiments in this study were performed with poly-L-lysine.HBr (PL-L) and poly-DL-lysine.HBr (PL-DL) as the adsorptives. As said before, polylysine was chosen because in solution this homopolyaminoacid shows different structures depending on the solution pH: at low pH, the molecule is a highly charged polyelectrolyte. At high pH the

molecule is helical, a structure which is also typical for proteins. Poly-DL-lysine does not form a helix and this polymer will behave as a coil at any pH.

As at low pH PL is highly positively charged, accurate concentration determinations are possible by means of titration with an oppositely charged polyelectrolyte.

As the adsorbents polystyrene (latex), pyrogenic silica and borosilica glass were used. For comparison reasons in some experiments also AgI was used. In this way it was possible to trace the influence of the nature of the surface of the adsorbent on the adsorption properties.

The present thesis is written in such a way, that each chapter can be read independently.

In chapter 2 some general aspects of the adsorption of ionizable polyaminoacids will be discussed.

In chapter 3 relevant properties of the polyaminoacids and adsorbents used obtained from the literature and from own measurements are discussed.

Chapter 4 deals with the adsorption of the highly charged polyelectrolyte polylysine on different substrates. The adsorbed amount as a function of time, molecular mass, ionic strength and surface charge is measured and discussed. Also the influence of the surface hydrophobicity is considered. Further the coagulation of the latex and sols used in the presence of an adsorbed PL layer are investigated.

In chapter 5 information is collected about the interaction between positively charged polylysine and negatively charged polystyrene (latex) and silica obtained with the help of conductometric and potentiometric techniques. At the same time the complex formation between polylysine and the solid particles is compared with the homogeneous complex formation between linear polycations and polyanions. The results are also related to the flocculation behaviour of the particles in the presence of PL.

Chapter 6 deals with the conformational properties of adsorbed PL at high pH. In the first part of this chapter interactions determining the secondary structure in PL are examined. As at low chain charge density phase separation occurs in PL solutions, some precipitation characteristics of PL-L and PL-DL are determined and the influence of the secondary structure of PL-L on the adsorbate properties is obtained from the comparison between the adsorption of PL-L and PL-DL as a function of the pH in the alkaline region.

By means of potentiometric proton titrations of PL-L and PL-DL in

solution and adsorbed on polystyrene, information is obtained about the PL conformation in the adsorbed state and the occurrence of conformational transitions in adsorbed PL. In the first part of the titration section, the application of proton titrations in heterogeneous systems is discussed in some detail. For comparison reasons also some titration experiments are performed with other polyelectrolytes. Finally the conformation of adsorbed PL as a function of the pH and adsorbed amount as it emerges from this study is roughly sketched.

In the last chapter some of the adsorption properties of PL are compared with the theoretical predictions of the polyelectrolyte adsorption theory of van der Schee.

1.4 REFERENCES

Cantor, C.R. and Schimmel, P.R. (1980). 'Biophysical chemistry' part I: The conformation of biological macromolecules. W.H. Freeman and Company, San Francisco. p. 235.

Daniels, S.T. (1980). 'Adsorption of microorganism to surfaces'. Bitton, G. and Marshall, K.C. eds. John Wiley & Sons, Chapter 2.

Fasman, G. (1967). 'Poly-α-Amino acids': Protein models for conformational studies'. Fasman, G. ed., Marcel Dekker Inc. New York.

Fleer, G.J. and Lyklema, J. (1983) in 'Adsorption from solution at the Solid/liquid Interface', Parfitt, G.D. and Rochester, C.H. eds., Academic Press, London p. 153-219.

Schee, H.A. van der (1984). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Norde, W. and Lyklema, J. (1978). J. Colloid Interface Sci. *66*, 285-293.

Norde, W. (1980). Adhesion and adsorption of polymers, part B. Lee, L.H. ed. Plenum Publ. Corp. New York, p. 801-825.

2 ADSORPTION OF POLYAMINOACIDS WITH IONIZABLE SIDE GROUPS

2.1 INTRODUCTION

This thesis is mainly concerned with the interaction between charged polyaminoacids (i.e. a class of polyelectrolytes) and oppositely charged colloidal particles and the conformational properties of the bound polyaminoacids. Following the definitions of *Bungenberg de Jong (1952)* the systems under consideration are complex colloid systems.

In the interaction between oppositely charged particles in aqueous solution, systems with increasing complexity can be considered.
In simple electrolyte solutions interactions such as ion pair formation between oppositely charged micro-ions are of importance. In homogeneous solutions of polyelectrolytes, the interaction between micro-ions and the polyion determines to a large extent the properties of the system. An example of current interest is the interaction of heavy metal ions with polyelectrolytes.
When two oppositely charged polyions are present in the solution, the complex formation between the oppositely charged polyions usually leads to phase separation. This type of complex was already studied extensively by *Bungenberg de Jong (1952)* for the gelatin/gum arabic system. Some 10 to 30 years later polyanion-polycation complexes were studied again with better defined polyelectrolytes (see chapter 5).

In sols or suspensions of charged solid particles, which are heterogeneous systems, the interaction between charged particles and oppositely charged polyelectrolytes is, as will be shown in chapter 5, to a large extent analogous to that between polyanions and polycations in homogeneous systems.

Of course also in the macromolecular systems, the interaction between the charged particles and the micro-ions is of importance. In the case of charged particles and oppositely charged polyelectrolytes, binding of the polyelectrolyte to the particle surface will usually occur.

Finally complex formation between oppositely charged solid particles in the absence or presence of polyelectrolytes can be considered. However this type of complex formation is beyond the scope of this study.

Table 2.1 Charge-Charge interactions in polyelectrolyte systems with oppositely charged macro-ions.

A	**Homogeneous systems**
1.	polyanion + micro-cation
2.	polycation + micro-anion
3.	polycation + polyanion
4.	micro-anion + micro-cation
B	**Heterogeneous systems with negatively charged interfaces**
1.	anionic interface + micro-cation
2.	polycation + micro-anion
3.	anionic interface + poly-cation
4.	micro-anion + micro-cation

The possible electric interactions between oppositely charged particles in polyelectrolyte systems are collected in table 2.1.

Except charge interactions also other interactions can play a role in the afore mentioned systems, such as hydrogen bonds and hydrophobic bonding. I will return to this and to the conformational aspects of the complex formation in the subsequent chapters.

2.2 POLYAMINOACIDS AT INTERFACES

In this section a short survey of literature concerning the adsorption of synthetic polyaminoacids is given.

E. Katchalski (1953) was one of the first who studied the adsorption of basic synthetic poly-α-aminoacids. These studies dealt with the interaction of basic polyaminoacids as polylysine, polyornithine and polyarginine with bacterial cell surfaces (*Katchalski et al., 1953*) and red blood cell surfaces (*Nevo et al., 1955*). These experiments were performed with polyaminoacids of a low degree of polymerization (DP 36-70). Results of these studies of a more general significance, in cases of charge contrast are:

- At a certain polyaminoacid concentration charge reversal occurs.
- Flocculation of the particles (i.e. cells) occurs just below charge neutralization.
- Electrostatic interactions are of major importance, but also non-electrostatic adsorption forces play a role.

The study of *Taketomi and Kuramoto (1978)* follows the same line as the early investigations of *Katchalski and Nevo*. These authors investigated the aggregation of human platelets by polylysine and dextran. Broadly outlined their results are analogous to those of *Nevo and Katchalski*. The interactions of polycations with mitochondria were investigated by *Tomasiak et al (1980)*. The effect of the polycations on the functioning of mitochondria was mainly considered. *Hartmann and Galla (1978)* investigated the binding of spin-labeled polylysine to charged bilayer membranes (i.e. binding to vesicles). A remarkable conclusion was that upon binding of highly charged PL a conformational transition from a random coil to a partially ordered conformation (**not** an α helix) may take place. It was further concluded that half of the lysine groups are bound to the membranes. However the results may be affected by the use of spinlabels.

As far as I know there are only a very few studies dealing with the adsorption of weak acid or basic homopolypeptides on solid substrates. *Corry and Seaman (1978)* and *Corry (1978)* studied the interaction between poly-L-lysine and polystyrene particles (latex) by means of electrophoretic mobility and aggregation studies. Results of their investigations of relevance for this study will be discussed in chapter 4.

In connection with the theory of chromatography on hydroxyapatite columns with small loads, *Kawasaki (1978)* studied the behaviour of low molecular mass poly-L-lysine in this type of adsorption chromatography.

Juriaanse et al. (1980a,b) investigated the adsorption of poly-L-lysine, poly-L-ornithine, poly-L-aspartic acid and poly-L-glutamic acid to whole Bovine Dental Enamel. Their results for the highly charged polylysine and polyornithine deviate from what is usually found for polyelectrolyte adsorption. The very high adsorbed amounts of about 20-30 $mg.m^{-2}$ (in the absence of added salt) are explained by these authors by an adsorption model in which only a few segments are attached to the surface and the repulsion between the resulting long tails is effectively screened by 'bridges' of divalent ions coming from the surface. The release of divalent ions was also experimentally verified. In my opinion also surface precipitation due to the binding of divalent ions can play a role. It will be clear that the results of *Juriaanse et al.* are not representative for simple solid-liquid interfaces. A study of the adsorption of acidic polyaminoacids to hydroxyapatite comparable to that of *Juriaanse et al.* was performed by *García-Ramos et al. (1981)*.

As already said in chapter 1 the study of charge-induced conformational transitions in an anisotropic environment is of prime interest in the study of adsorbed polyaminoacids. Nevertheless little attention has been paid to this subject in the literature.

Although *Caspers et al. (1974)* studied the conformation of an ionizable polypeptide adsorbed at the air-water interface and not at solid-liquid interfaces, their result is worth mentioning here. *Caspers et al.* found that a charge-induced transition from the helical- to the coiled conformation is possible in a copolymer of glutamic acid and methyl glutamate adsorbed at the air-water interface.

Especially with respect to the conformational properties, the polypeptide behaviour at solid-liquid and liquid-fluid interfaces can be different. For example, at liquid-fluid interfaces sterical constraints may be less important than at solid-liquid interfaces, as a consequence of which conformational transitions may occur more easily.

Pefferkorn et al. (1982) studied the helix-coil transition of polyglutamic acid adsorbed at an artificial (solid) membrane surface. The influence of the polyglutamic acid conformation on the membrane properties was investigated. Their results indicate that a helix-coil transition in the adsorbed state occurs indeed when the solution pH is changed over a certain region.

A study closely related to the underlying one is that of *van der Schee and Lyklema (1982)*, who studied the adsorption behaviour of oligo- an polyaminoacids on AgI and their effects on double layer properties and colloid stability.

The dimensions and states of uncharged polypeptide molecules adsorbed at solid-liquid interfaces were investigated by *Chao (1974)*. This author measured the adsorption of a variety of uncharged polyaminoacids from various solvents on glass. The discussion of his results is beyond the scope of this thesis.

Discussions about the properties of polyaminoacids adsorbed or spread at liquid-liquid or liquid-air interfaces can be found in the reviews of *Miller (1971)*, *Miller and Bach (1973)* and *Malcolm (1973)*.

2.3 POLYELECTROLYTE ADSORPTION

The adsorption of polyelectrolytes on solid substrates is more complex than that of uncharged polymers, because of the electrostatics of the polymer/adsorbent and polymer/polymer interaction.

In general, polyelectrolytes can become adsorbed on adsorbents op-

positely charged to the polyelectrolyte (then ion exchange phenomena may play a role) (see *Hesselink, 1977*), but also on adsorbents with the same type of charge as the polyelectrolyte. The latter occurs only when the non-electrostatic adsorption energy is high enough to overcome the electrostatic repulsion.

Usually high affinity adsorption isotherms are found. In most cases the amount adsorbed is lower than that for non-ionic polymers due to the strong repulsion between segments in the adsorbed layer. At high ionic strength, or at low chain charge density, polyelectrolyte adsorption should resemble non-ionic polymer adsorption. It seems therefore logical to take the train-loop-tail model (as in theories for uncharged polymer adsorption) as a starting point for the description of flexible polyelectrolyte adsorption. Whether such an description is also valid for polyelectrolytes showing secondary structure is questionable (see chapter 7).

The adsorption of polyelectrolytes on solid adsorbents is mainly dependent on:

- Properties of the adsorbent; e.g. surface charge density σ_o, surface heterogeneity, hydrophobic/hydrophilic balance of the surface.
- Properties of the polyelectrolyte; e.g. degree of polymerization DP, pK's of the charged groups, spacing between the charges, chain flexibility.
- Interaction between polyelectrolyte and solvent.
- Interaction between polyelectrolyte and adsorbent as determined by the effective adsorption energy $\chi_{s,eff}$, defined as the non-electrostatic adsorption energy χ_s including the electrostatic interactions of train segments with the surface.
- Properties of the solution, e.g. ionic strength, polymer volume fraction, pH, presence of bivalent ions, presence of specifically adsorbed (i.e. through non-electrostatic forces) ions.

In a theoretical treatment on polyelectrolyte adsorption it would be a formidable task to include all these contributions. In the existing theories the independent variables which are taken into account are: polymer concentration in solution, chain length, surface charge σ_o, polymer chain charge density, the Flory-Huggins polymer-solvent interaction parameter χ and the non-ionic adsorption energy parameter χ_s. For a discussion of experimental and theoretical trends the reader is referred to the review by *Hesselink (1983)*. However, more recent (i.e. after 1979) experimental and theoretical developments are not

included in Hesselink's article. Some of these if of importance for this study, can be found in chapter 4 to 7.

The first theory on polyelectrolyte adsorption, capable of describing some experimental trends was that of *Hesselink (1977)*. However this theory is nevertheless inadequate because some important properties are predicted incorrectly. For example, the fraction of segments p attached to the surface and the predicted thick polyelectrolyte layers are greatly in disagreement with general experimental experience.

Usually, high p values and thin adsorbed layers are found, at least at low ionic strength.

A model for the adsorption of polyelectrolytes analogous to that of *Hesselink*, was published by *Silberberg (1978)*.

Recently, *van der Schee (1984)* extended the theories of *Roe (1974)* and *Scheutjens-Fleer (1979)* to the adsorption of charged flexible polymers. *Van der Schee* derived expressions for the potential distribution in and the free energy of a double layer containing polyelectrolyte charge. In the model of *van der Schee* cilindrical symmetry, which is usually encountered in theories for bulk polyelectrolyte, is lost. In contrast to the theory of *Hesselink*, the polymer segment-density as a function of the distance to the surface follows from the theory and is not preassumed as *Hesselink* did (see also chapter 7).

At low electrolyte concentration the theory does predict the high values of p and thin polyelectrolyte layers. Also other properties are predicted correctly, for example the ionic strength dependence of the adsorption and the effect of molecular mass. However some other properties of adsorbed polyelectrolyte predicted by the theory of *van der Schee* may be at variance with experimental observations due to the approximations done in the theory. The most serious of these are:

- The use of a lattice model for adsorbed and bulk polyelectrolyte in which the characteristic length for the polyelectrolyte segments is the same as for the electric interactions.
- The smearing out of charges in planes parallel to the surface, also for the description of bulk polyelectrolyte.
- The neglect of chain stiffness effects.
- The neglect of the influence of the potential on the dielectric constant and the degree of ionization.

The consequence of these approximations, which except for the last mentioned, are difficult to avoid at present, is that it is very likely that proton titration properties of adsorbed polyelectrolyte will not

be predicted well. A discrepancy between theory and experiment is also expected when discrete charge effects at the surface are important. This may be the case with adsorbents that have localized ionizable groups at the surface.

Nevertheless, the polyelectrolyte adsorption theory of *van der Schee* is a fruitful development in the polyelectrolyte adsorption field, warranting further elaborations.

2.4 REFERENCES

Bungenberg de Jong, H.L. (1952) in 'Colloid Science', Kruyt, H.H., ed. Amsterdam, vol. 2.

Caspers, J., Berliner, C., Ruysschaert, J.M. and Jaffe, J. (1974). J. Colloid Interface Sci. *49*, 433-441.

Corry, W.D. and Seaman, G.V.F. (1978). J. Colloid Interface Sci. *63*, 136-161.

Corry, W.D. (1978). J. Colloid Interface Sci. *63*, 151-160.

Garcia-Ramos, J.V., Carmona, P. and Hidalgo, A. (1981). J. Colloid Interface Sci. *83*, 479-484.

Hartmann, W. and Galla, H.J. (1978). Biochim. Biophys. Acta *509*, 474-490.

Hesselink, F.Th. (1977). J. Colloid Interface Sci. *60*, 448-466.

Hesselink, F.Th. (1983) in 'Adsorption from solution at the Solid-liquid Interface', Parfitt, G.D., Rochester, C.H., eds. Acad. Press London.

Juriaanse, A.C., Arends, J. and Ten Bosch, J.J. (1980). J. Colloid Interface Sci. *76*, 212-219.

Juriaanse, A.C., Arends, J. and Ten Bosch, J.J. (1980). J. Colloid Interface Sci. *76*, 220-226.

Katchalski, E., Bichowski-Slomnitzki and Volcani, B.E. (1953). Biochem. J. *55*, 671-680.

Kawasaki, T. (1978). J. Chromatogr. *157*, 7-42.

Malcolm, B.R. (1973) in 'Progress in Surface and Membrane Science' *7*, Danielli, J.F., Rosenberg, M.D. and Cadenhead, D.A., eds. Acad. Press, New York-London, p. 183-229.

Marra, J., Schee, H.A. van der, Fleer, G.J. and Lyklema, J. (1983) in 'Adsorption from Soltuion', Ottewill, R.H., Rochester, C.H. and Smith, A.L., eds. Acad. Press 245-258.

Miller, I.R. (1971) in 'Progress in Surface and Membrane Science' *4*, Danielli, J.F., Rosenberg, M.D. and Cadenhead, D.A., eds. Acad. Press, New York-London, p. 299-350.

Miller, I.R. and Bach, D. (1973) in 'Surface and Colloid Science' *6*, Matijević, E., ed. J. Wiley & Sons, p. 185-260.

Nevo, A., de Vries, A. and Katchalsky, A. (1955). Biochim. Biophys. Acta *17*, 536-547.

Pefferkorn, E., Smith, A. and Varoqui, R. (1982). Biopolymers *21*, 1451-1463.

Roe, R.J. (1974). J. Chem. Phys. *60*, 4192-4207.

Schee, H.A. van der and Lyklema, J. (1982) in 'The effect of polymers on dispersion properties', Tadros, Th.F., ed., Acad. Press, London, p. 81-100.

Schee, H.A. van der (1984). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Silberberg, A. (1978) in 'Ions in Macromolecular and Biological Systems' (Colston Papers *29*), Everett, D.H. and Vincent, B., eds., Scientechnica Bristol, p. 1-10.

Scheutjens, J.M.H.M. and Fleer, G.J. (1979). J. Phys. Chem. *83*, 1619-1635, *84* (1980), 178-190.

Taketomi, Y. and Kuramoto, A. (1978). Thrombos, Haemostas. (Stuttg.) *40*, 11-23.

Tomasiak, M., Tomasiak, A. and Rzeczycki, W. (1980). Bulletin de l'Academie Polonaise des Sciences (serie des sciences biologiques) CLII, vol. *28*, 1-6.

3 MATERIALS

3.1 POLYLYSINE (PL) AND RELATED POLYAMINOACIDS

3.1.1 Synthesis and structure

The polyaminoacids used in this study were commercially produced by the Sigma chemical company. Their viscosity average molecular mass, as determined by the manufacturer, were used in this study. The general primary structure is shown in *fig. 3.1*.

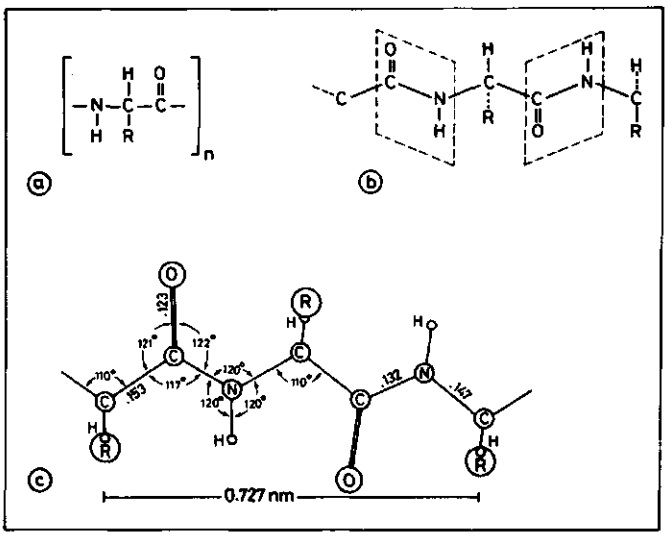


Fig. 3.1

a. Repeating chemical unit of a polyaminoacid chain.
R = $-(CH_2)_3-NH_3^+ Br^-$, polyornithine.HBr.; R = $-(CH_2)_4-NH_3^+Br^-$, polylysine.HBr.

b. Planar peptide bond unit $-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-$ in a polyaminoacid chain, characteristic for polypeptides and proteins.

c. Dimensions and configuration of a fully extended polypeptide chain (after L. Pauling et al. (1951). Dimensions are given in nm.

Different homopolyaminoacids differ with respect to the composition of the side group R (fig. 3.1) of the polypeptide backbone. The nature of this side group is very important in determining the solvent proper-

ties and secondary structure.

The polyaminoacids are prepared by base initiated polymerization of N-carboxyanhydrides of the corresponding α-aminoacids. This results theoretically in a Poisson-type distribution for the molecular mass of the molecules. The poly-DL-lysine samples used are atactic. The viscosity average molecular mass of each basic polyaminoacid has been determined by the manufacturer from the solution viscosity of the material using the poly-L-lysine calibration method described by *Yaron and Berger (1963)*, *Sigma (1980)*. The molecular mass of poly-L-glutamic acid (PGA) used in some experiments has been determined also by the manufacturer from the solution viscosity of the polyaminoacid following *Idelson and Blout (1958)*.

Zimmerman and Mandelkern (1975) found for a PGA sample from Sigma ($\bar{M}_v$ 102.680) a $\bar{M}_w/\bar{M}_n$ ratio of 1.13, which is to be expected for a Poisson type distribution. These authors found also that the absolute value of the molecular mass was always appreciably lower (10-20% for PGA samples from Sigma) than the values given by the supplier. It is possible that this is also the case for the PL samples used here. It is very likely that the $\bar{M}_w/\bar{M}_n$ ratio is also close to 1.0 for the polyaminoacid samples used in this study.

Most of the experiments were performed with polylysine, here the side chain group R (see fig. 3.1) is $-(CH_2)_4-NH_3^+Br^-$. Some experiments were done with polyornithine R = $-(CH_2)_3-NH_3^+Br^-$, polyhistidine R = $-CH_2-$  and the sodium salt of polyglutamic acid R = $-(CH_2)_2-COO^-Na^+$. Some PL samples were checked for residual blocking groups by UV, IR and NMR spectroscopy. In most cases there was no evidence found for the presence of such impurities. The water content of the PL samples appeared to be 2-4% after drying under vacuum over KOH. The Br^- content of the PL.HBr samples as determined from the weighted amount was in good agreement with the value found from conductometric or potentiometric titration of a PL.HBr solution with a calibrated $AgNO_3$ solution. In most experiments the polyaminoacid samples were used as received.

3.1.2 Solution properties of PL

Most homopolyaminoacids are not soluble in water. Of the uncharged polyaminoacids only polyproline and polyhomoserine are soluble in water (*Fasman, 1967*). Poly-DL-alanine has a limited solubility in water, while poly-L-alanine is not soluble at all in water (*Doty and Gratzer, 1961*). This is probably caused by the impossibility of the formation of

intramolecular hydrogen bonds in its molecule. The salts of polyaminoacids with an ionizable side group are also soluble in water or aqueous low molecular mass electrolyt solutions. However, the uncharged basic or acidic polyaminoacids are insoluble in aqueous electrolyte solutions. Hence for these polyaminoacids in water the well known Flory Huggins interaction parameter χ exceeds 0.5. There are no data concerning the measurement of the χ parameter of basic (PL and PO) or acidic (PGA) polyaminoacids available from literature. Interpretation of colligative and hydrodynamic properties of polyelectrolytes in terms of a second virial coefficient, from which χ values are derived in the case of non-ionic polymers, is very much complicated by the presence of electric charges. So the usual techniques for determining χ values are not suitable yet for polyelectrolytes.

Some information concerning the Flory-Huggins interaction parameter may be obtained from phase-separation data, because phase separation occurs near charge neutralization of the polyelectrolyt. However, the occurrence of secondary structure can complicate the precipitation behaviour of polyelectrolytes and polyaminoacids (*Zimmerman and Mandelkern, 1975*). Measurements on the precipitation behaviour of PL will be reported in Chapter 6.

As stated in section 1.2, one of the attractive features of synthetic polyaminoacids is that their secondary structure can be manipulated by varying experimental conditions. For polyaminoacids with ionizable side chains a change in secondary structure (helix-coil transition) can occur when the charge density on the polypeptide chain is changed by adjusting the pH of the aqueous solution (*Fasman, 1967*). For PL-L these changes in secondary structure can very nicely be followed with CD spectroscopy, see for example *Beychok (1967)*. The CD spectra of poly-L-lysine in the α, β or coil conformation are even used as standard for the estimation of the helix and β sheet content of proteins (*Greenfield and Fasman, 1969*). The CD spectra of a PL-L sample from Sigma used here shared the same characteristics at pH 6 and 11.0 as those reported in the literature. CD measurements with poly-DL(1-1)-lysine cannot reveal any indications of helices at any pH, since poly-DL(1-1)-lysine is in fact a racemic mixture of L and D lysine residues.

With potentiometric proton titrations however it is possible to monitor conformational changes both in poly-L-lysine and poly-DL-lysine as a function of the charge density on the polylysine chain.

Potentiometric proton titration data of weak acid or basic polyelectrolytes contain information about the conformation of the macromolecule because the measured pK (= pH-log $\frac{\alpha}{1-\alpha}$) contains an electrostatic term which is dependent on the electric field on the polyion chain, and hence depends on the conformation of the molecule. The 'intrinsic' pK_o value at a fixed polyion- and salt concentration can be found by extrapolating the apparent pK values to zero charge density on the chain. (*Nagasawa, 1970*). More details about the method can be found in section 6.6. The apparent pK (= pH-log $\frac{\alpha}{1-\alpha}$) as a function of the degree of dissociation is plotted in fig. 3.2 for poly-L-lysine and poly-DL-lysine in 0.1 M NaBr. The curve for poly-L-lysine shows a 'dip' around pH 10, caused by the coil to helix transition. As one can see this 'dip' is absent in the PL-DL curve. So the PL-DL molecule shows as expected no coil to helix transition, and thus stays in a coil conformation at pH values were PL-L is helical. The curve found for PL-L is in reasonable agreement with those reported in literature (e.g. *Hermans, 1966; Grourke and Gibbs, 1971*). See also Chapter 6.

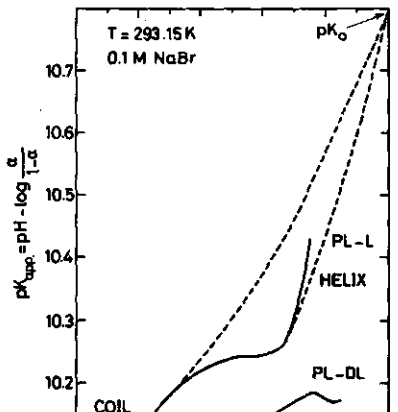


Fig. 3.2 Proton titrations of poly-L-lysine and poly-DL-lysine.
$c_{PL-L} = 2.81\ mol_r.m^{-3}$; $c_{PL-DL} = 4.87\ mol_r.m^{-3}$.
See also fig. 6.12.1 and fig. 6.13 in Chapter 6.

The coil conformation of PL-L and PL-DL at acidic pH values are probably not the same. The characteristic ratio for the racemic random poly-DL-aminoacid is about a factor of two lower than that of the corresponding poly-L- or poly-D-aminoacid (*Flory, 1969*). This corresponds to a more flexible chain for the poly-DL-aminoacid. At low ionic strengths the effect of the charges on the polyaminoacids on the chain stiffness is dominant. *Van der Schee (1981)* found a strong rise of the optical rotation of L-lysine oligomers in water (pH<7) with increasing chain length up to 32 residues. This is indicative of a structure developing gradually to lengths of more than 10 residues. Other authors found also evidence for the occurrence of optically active structure in poly-L-lysine molecules in water at low pH values (coil). Circular dichroism measurements have pointed to an extended helix (*Tiffany and Krimm, 1969, 1972; Rippon and Walton, 1971*), although this idea has been contradicted among others by *Balasubramanian (1974). Painter and Koenig (1976)* contributed to this dispute with their Raman and IR spectroscopic study of the solution conformation of poly-L-lysine. Their results are in favor of the presence of some local ordered structure at low ionic strength similar to that suggested by Tiffanny and Krimm. CD and optical rotation measurements are not suitable to detect the presence of some structure in random (1:1) poly-DL-lysine because solutions of these molecules are racemic mixtures of L- and D residues as stated earlier. Hence, with the above mentioned techniques it is not possible to compare experimentally the flexibility of poly-L- and poly-DL-lysine molecules.

Information about the chain stiffness may be obtained from for example viscosimetric, light scattering and flow birefringence measurements. The determined chain stiffness at low ionic strength will be large due to electrostatic effects, i.e. the electrostatic persistence length, which is identical for PL-L and PL-DL is the largest part of the total persistence length. Above ionic strengths of 0.1 M and at acidic pH differences in chain stiffness due to different stereoregularity become visible, because then the $-NH_3^+$ charges of PL are screened. This difference in chain stiffness can cause a different adsorption behaviour of poly-L- and DL-lysine at higher salt concentrations.

To conclude this section fig. 3.3 shows molecular models of a part of a poly-L-lysine chain in the fully extended, and compact conformation.

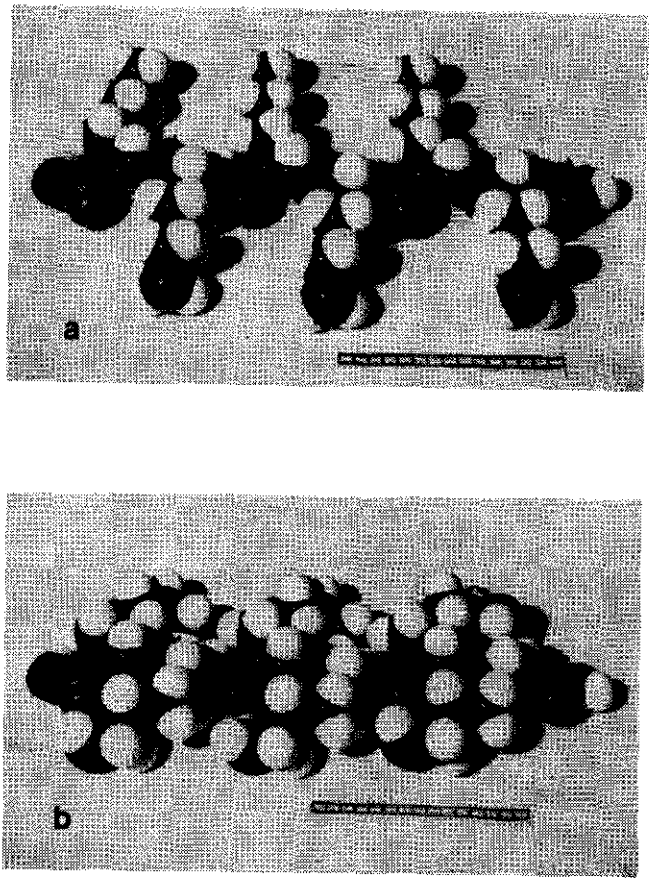


Fig. 3.3 Stuart models of poly-L-lysine. a) extended configuration, b) compact conformation.

3.2 POLYSTYRENE LATEX

3.2.1 Preparation

The preparation and characterization of emulsifier-free polystyrene

latices was essentially the same as described by *Furusawa et al. (1972)* and *Norde (1976)*, except for two modifications in the purification step: After preparation of the latex (latex M: $c(K_2S_2O_8)$ = 1.85 mM, $c(KHCO_3)$ = 10 mM; Latex L: $c(K_2S_2O_8)$ = 0.31 mM), the 4-6 latex samples of about 200 cm^3 (solid content: 6-8% (w/v)) obtained in one latex preparation run, were mixed together when the particle diameter of each sample was the same within narrow limits. Then this latex batch was steamstripped under reduced pressure at 308-313K, to remove excess monomer. During this process the ionic strength of the latex was kept at the same or at a lower level than the initial value. Then the latex was treated batchwise with a tenfold excess ion exchange resins from Biorad (Dowex 50W-x_4 cationic resin, Dowex 1-x_4 anionic resin), using a mixed bed technique. The tenfold excess is with respect to initial buffer value (i.e. ionic strength) of the latex. In this way the low molecular mass salts were removed and the latex was brought into the protonated form. Conductometric titration of the latex after the first and a second treatment with the ion exchange resins showed that one run of ion exchange sufficed. The ion exchange resins were extensively purified following the method of *Van den Hul and Vanderhoff (1968)*. After the ion exchange treatment and determination of the surface charge of the latex, the latex was brought into the Na^+ form by titration with NaOH and stored in a refrigerator at 276 K until use, to slow down any surface group hydrolysis and microbial growth.

3.2.2 Characterization of PS latex

Properties of a latex, like particle diameter, number and nature of surface groups etc., are in first instance dependent on the conditions prevailing during the polymerization reaction. For instance, the surface charge density can be varied by varying the initiator concentration and the concentration of buffer and/or supporting electrolyte. For a detailed description of the influence of polymerization conditions on the latex properties I refer to the review by *Hearn et al. (1981)*. The properties of the prepared PS latex can be altered by the cleaning procedure applied and the duration and conditions of latex storage (*see also Hearn et al., 1981; van den Hoven, 1984*). In the beginning of this study dialysis of the latex was used to remove the low molecular mass salts and unreacted styrene monomer in the same way as described by *Furusawa et al. (1972)* and *Norde (1976)*. Because of the possible contamination of the latex during the prolonged dia-

lysis and the ineffectivity of this process (*Hearn et al., 1981*), the dialysis step was skipped and replaced by steamstripping and an ion exchange procedure to remove excess electrolyte and to bring the latex in the protonated form as already described under 3.2.1. For studies on protein adsorption on PS latex, *Norde (1976)* used latex which was only dialysed, because use of ion exchange resins would possibly contaminate the latex by polyelectrolytes leaching from the resin, even after extensive purification of the resins. This has indeed been found by *McCarvill and Fitch (1978)* when cation- and anion exchange resins are used separately. On the other hand, when mixed bed resins are used *McCarvill and Fitch (1978)* and *VanderHoff (1970)* found no indication of surface contamination. The reasons for this apparent contradiction are not clear (*Hearn et al., 1981*). The use of ion exchanged latex for adsorption studies has the advantage over dialysed latex of a more accurately known surface charge.

Knowledge of the surface charge of the latex requires besides the titration charge of ion exchanged latex, at least additional information about the sulphur content of the latex before and after ion exchange (*Norde, 1976*). A detailed discussion of the advantages and disadvantages of the various latex cleaning procedures can be found in the review by *Hearn et al. (1981)*. Despite possible differences in latex properties by applying different cleaning procedures there were no indications found in this study that the adsorption behaviour of polylysine on latex is very dependent on the cleaning procedure adopted. Adsorption isotherms of PL on only dialysed latex or steamstripped and ion exchanged latex were within experimental accuracy the same. The same was found for proton titrations of adsorbed polylysine.

The mean particle size of PS particles was determined by transmission electron microscopy. Operating conditions were selected such to minimize the effects of the electron beam on the particles. The instrument was calibrated at the magnifications used against a carbon replica of a diffraction grating. All samples used showed monodisperse spherical particles. The mean particle size of the PS particles was obtained from EM pictures by measuring the particle diameter of 25-90 particles, in the same way as described by *Norde (1976, 1978)*.

The uniformity coefficient U, defined as $U = d_w/d_n$, where d_w and d_n are the weight and number average particle diameter respectively, is a measure of the homogeneity of the latex. d_w/d_n can be calculated from the individual particle diameters d_i using:

$$d_w/d_n = \{(\Sigma_i n_i d_i^6)/(\Sigma_i n_i d_i^3)\}^{1/3} \; / \; \{(\Sigma_i n_i d_i)/\Sigma_i n_i\} \qquad (3.1)$$

where n_i is the number of particles. For all negatively charged PS latices used the size distributions are very narrow, with $1.000<U<1.01$. See also table 3.1. From this table one can also see that with the same latex formulation it is possible to obtain latices with the same particle diameter with a reasonable degree of reproducibility. The surface charge of the latices however, prepared with exactly the same formulation is not so well reproducible (see below and table 3.1).

Table 3.1 Relevant properties of PS latices used in this study

PS Latex	$\bar{d}$ (nm)	U	surface conc. $-OSO_3^-$ (μmol.g^{-1})	A_{sp} (EM) $m^2.g^{-1}$	A_{sp} (MB) $m^2.g^{-1}$	σ_o mC.m^{-2}
M_1	613 ± 19	1.005	6.99	9.3		-74
M_2	551 ± 14	1.003	5.98	10.4		-60
M_3	657 ± 30	1.008	5.0	9.2	4.0	-52
M_4	602 ± 21	1.005	4.2	10.0		-40
M_5	622 ± 16	1.003	3.6	9.2		-39
L	488 ± 18	1.006	1.3	11.7	1.7	-11
P	405 ± 35	1.03	-	14.8		+

Another observation made by *van den Hoven (1984)* is that the latex particle diameter is lowered by ~3%, after the ion exchange treatment. A possible qualitative explanation for this phenomenon may be that a complete layer of oligo and polystyrene molecules is stripped of from the latex surface. This is in accordance with the loss in bulk sulphur content of the latex particles up to 30% observed by *Norde (1976)*. Another possibility is that fractionating of the particles occurred due to the ion exchange treatment. It is also possible that the PS particles have another behaviour in the electron beam after ion exchange. However, the effect on the specific surface area of the latex is within experimental error in our case. Because of the monodispersity of the latex samples, the specific surface area A_{sp} (surface area per gram polystyrene) may be simply calculated using:

$$A_{sp} = \frac{6}{\rho.\bar{d}} \qquad (3.2)$$

in which ρ is the density of polystyrene, where it does not matter whether

d_w or d_n is chosen for d. *Norde (1976)* found by means of pyknometry, densities of the PS latex particles between 1,048 and 1,052 kg m^{-3} The specific surface area found with (3.2) is the geometric surface area, so the actual (physical), surface area, which depends on the method used for its determination, may be higher due to surface irregularities ('hairy' surface) and for porosity of the latex surface. There are indications that the N_2 B.E.T. specific surface area determined with freeze dried PS latex samples is higher (up to 30%) than the EM surface (*Norde*, personal communication). *Barclay (1970)* reported a similar result. *Kamel (1981)* however found almost no differences between the two surface areas. Because of their shape the surface 'seen' by macromolecules may be closer to the geometrical one than to the B.E.T. (N_2) specific surface area.

The adsorption of low molecular mass compounds from solution is a common and experimentally relatively easy technique for estimating the interfacial area of adsorbents at the solid-liquid interface.

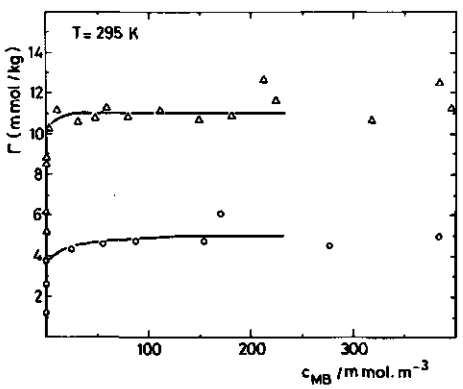


Fig. 3.4 Adsorption isotherms of MB on PS latex. T = 295 K
o-o PS latex M_3 σ_o = 120 C.kg^{-1}; pH(ads) ≃ 5.5; A_{sp}(geom.) = 9.2 m^2.g^{-1}
Δ-Δ PS latex L σ_o = 500 C.kg^{-1}; pH(ads) ≃ 5.5; A_{sp}(geom.) = 11.0 m^2.g^{-1}.

In fig. 3.4 adsorption isotherms of methylene blue (MB) from aqueous solution on PS latices with different surface charge are shown. The adsorbed amount of MB was determined by depletion from solution, re-

moving the PS particles in essentially the same way as for PL adsorption (section 4.2.3). The MB concentration was determined spectrophotometrically at a Beckman model 3600 spectrophotometer at 663 nm. The MB used was analar grade from Baker.

The adsorption isotherm of MB is a high affinity one. This was also found for the adsorption of MB on AgI (*Koopal, 1978*). The plateau value is very sensitive to the surface charge of the latex as one clearly can see from fig. 3.4. When in both cases monolayer adsorption at the plateau adsorption is assumed and for the pH under consideration, a cross section a_o per molecule MB of 0.55 nm^2 (monolayer of MB dimers) is taken (*Koopal, 1978*) then the MB surfaces of latex M_3 and L are respectively 4.0 m^2g^{-1} and 1.7 m^2g^{-1}, whereas the specific surface areas obtained from EM are 9.2 and 11.0 m^2g^{-1} respectively. Even if MB monomer adsorption should occur the values found for A_{sp} are lower than the geometrical ones. It seems that only the charged groups on the latex surface and their direct environment are possible adsorption sites (patchwise adsorption). Coagulation of the latex after charge neutralization by MB occurred. In principle this can lead to a surface area reduction and the estimated MB plateau adsorption values may be somewhat too low.

From the above the conclusion may be drawn, that MB adsorption is not suitable for specific surface area measurements of latices, but rather a crude measure for the surface charge of a latex. A similar conclusion was reached for the adsorption of MB on silica and glass (see section 3.3.1 and 3.3.2).

Kasper (1971) used the complexation of MB with the surface sulphonate groups for the determination of the surface charge of the latex he used for flocculation studies. As the adsorption of MB is superequivalent, the values he obtained are too high. It may be noted that there is a reasonable agreement between the MB, N_2 B.E.T. and geometrical surface area of AgI suspensions (*Koopal, 1978; de Keizer, 1981*). The mobility of the surface charges and/or adsorbate molecules on AgI may be responsible for this.

In connection with the above, the adsorption of tetra alkylammonium ions (TAA^+) on solid-liquid interfaces, might also be considered. These ions, have a spherical shape, so that no problems arise in determining the orientation at the interface. The cross section of a TAA^+ ion at the interface is thus well defined. *De Keizer (1981)* found for the tetrabutylammonium area of AgI suspensions a value that is 10-20% higher than the MB surface area. This difference is within expe-

rimental error. However the plateau values of tetrapropyl and tetrabutyl ammonium ions are the same, indicating that TAA^+ ions do not form a dense monolayer on AgI, and therefore they give a too low value for A_{sp}. A similar situation exists for PS latex.

Van den Hoven (1984) measured the adsorption of tetrapropyl ($TprA^+$), tetrabutyl ($TBuA^+$) and tetra-amylammonium ($TAmA^+$) ions on PS latex (σ_o = 82.9 mC m^{-2}). The affinity of the TAA^+ ions for the latex increases in the given order. However the plateau value is the same for all three TAA^+ ions: 0.8 μmol m^{-2}. This value corresponds well with the surface charge of the latex, indicating that the TAA^+ ions adsorb only at charged ($-OSO_3^-$) groups on the surface. The ionic radius of TAA^+ increases in the order $TprA^+$, $TBuA^+$, $TamA^+$, so the calculated apparent specific surface area increases also in the given order. The TAA^+ area is in all cases significantly lower than the geometrical surface area. In this respect TAA^+ ions behave similar to MB ions at pH 5.5. The observations above are of importance for the interpretation of the plateau adsorption value of polylysine.

The surface charge of the PS latices was determined after ion exchange by conductometric and/or potentiometric titration with 0.1 M NaOH under N_2 atmosphere. For details of the titration method see section 4.2.4. There were no significant differences between the equivalence points of the titrations after a first and a second treatment with ion exchange resin, indicating that after the first treatment protonation was complete. In fig. 3.5 a typical example of a conductometric titration is shown.

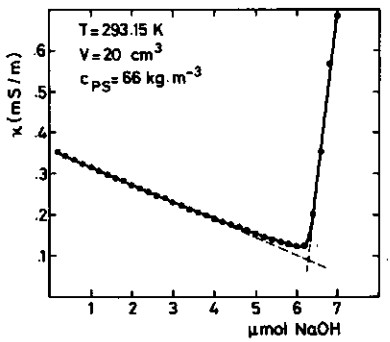


Fig. 3.5 Conductometric titration of ion exchanged (mixed bed) PS latex M_3 (H^+ form) with 0.1 M NaOH.

From the titration curves it was conducted that the latices used did not contain significant amounts of weak acid groups. The presence of surface OH groups (resulting from the Kolthoff reaction during polymerization, or hydrolysis of $R\text{-}OSO_3^-$ groups during storage) is of course not excluded. However the Kolthoff reaction is suppressed due to the use of $KHCO_3$ in the reaction medium (*Hearn et al., 1981*). The slope of κ vs $[OH^-]$ (see fig. 3.5) before the equivalence point is appreciable lower (4-5 fold) than the slope for the corresponding strong acid titration, as is generally observed for PS latex titrations. In part this is due to a lower contribution to the overall conductivity of H^+ ions in the electrical double layer. With linear polyelectrolytes a similar behaviour is observed.

Another question is whether or not the surface charge of PS latex is the same before and after ion exchange. *Norde (1976)* assumed that the reduction in total bulk sulphur that he found after ion exchange was completely attributable to the loss of oligo- and polystyrene sulphonate molecules during the exchange process, and that the burried bulk sulphate groups are not exposed at the interface after ion exchange. He assumed then that the surface charge (Cg^{-1}) before ion exchange is the measured one after exchange plus the difference in sulphur content before and after the ion exchange treatment per gram latex. For PS latex M_1 (i.e. a latex with the same formulation as used here), he found a σ_o value (after ion exchange) of -45 mCm^{-2} and σ_o (before i.e.) = -84 mCm^{-2}. There are several facts that plead against a reduction in σ_o during the ion exhange process:

(i) repeated ion exchange treatments give the same value of σ_o.

(ii) The adsorption plateau value of PL on PS latex M is the same before and after exchange, while the plateau value is sensitive to the surface charge at least in the region -(10-60) mCm^{-2} (see section 4.3.5).

An alternative explanation is that due to the ion exchange treatment a thin layer polystyrene is stripped off, thus forming a new PS surface with new $-OSO_3^-$ groups, who where burried as ion pairs in the PS particle before the treatment. The consequence then is that the σ_o values given by *Norde (1976, 1978)* for latex before ion exchange may be about 50% too high.

Latex that once has been exposed to 0.1 M electrolyte solution can have a surface charge that is appreciable higher (sometimes by as much as a factor two) than latex that has not been at high (>0.1 M) salt concentrations. This was concluded by *van den Hoven (1984)* from conductometric titrations of latex samples who were ion exchanged before

and after exposure to 0.1 M KNO_3. When a second exposure to 0.1 M KNO_3 was applied no increase in σ_o was observed. No clear interpretation of this salt effect can be offered at the moment. The latices used in this study were not treated with 0.1 M electrolyte solution, before use in adsorption experiments, except for latex M_1. The consequences of the uncertainty in σ_o at high ionic strength will be discussed in sections 4.3.5, 4.3.6 and 6.6.4.
The question whether the surface of the PS particles is smooth or 'hairy' will be dealt with in the next section.

3.2.3 The polystyrene-water interface

In sections 3.2.1 and 3.2.2 some properties of the PS latices were discussed, related to the specific surface area, nature and amount of surface groups. In this section some other properties of the PS-water interface will be discussed that may be of relevance for the adsorption of polyaminoacids.

As stated in the previous section, on the surface there are mainly $-OSO_3^-$ groups. For a latex with a surface charge of, say, -50 mCm^{-2}, the total surface area of these groups is 0.15 m^2 per m^2 geometrical surface area. The rest of the surface is hydrophobic. Thus, the surface 'seen' by positively charged adsorbates like MB, TAA^+ ions and polylysine is very likely to be heterogeneous in nature.

Another consideration is whether the polystyrene surface is smooth or 'hairy'. By 'hairy' it is meant, that the surface is covered with bound oligo- and polystyrene molecules dangling in solution.

Goossens and Zembrod (1981) found an increase in particle diameter of a carboxylated latex with photon correlation spectroscopy upon increasing the pH from 3 to 8. This is expected in the case of a 'hairy' surface on which the 'hairs' consist of polymer chains with carboxylate groups. The polymer chains are soluble in water because of the charged end groups. Uncharged polystyrene molecules are nearly unsoluble in water ($\chi > 0.5$) and will therefore lay more flat on the surface, thus forming a more or less 'smooth' surface.

From electrokinetic measurements on PS latex plugs *van den Hoven (1984)* found evidence that also latices with $-OSO_3^-$ groups as used here show a 'hairy' character. The surface charge of these latices however, is much lower than that of the carboxylated latices of Goossens and Zembrod. The hairy layer is therefore probably less dense than in the case of a highly charged latex.

The hydrophobicity of the polystyrene -water interface is an important factor determining the adsorption properties of adsorbates with hydrophobic groups such as the $-(CH_2)_4-$ group in the side chain of a PL residue, because of the possibility of hydrophobic interactions between these groups and the adsorbent.

Since the polystyrene itself is hydrophobic but the $-OSO_3^-$ groups and also -OH, -COOH and specifically adsorbed ions, if any, are hydrophilic in nature, the polystyrene-water interface consists of hydrophilic patches in an otherwise hydrophobic environment. The density of these polar groups on the surface determines the overall hydrophobicity, but the meaning of that for adsorption properties is obscure.

Because of the patchwise distribution of hydrophobic regions, the state of hydration varies also along the surface. The state of hydration along a polypeptide chain is very important in determining the secondary and tertiary structure of these molecules. Because of this it is very likely that the state of interfacial water plays a role, in determining the structure of adsorbed polypeptides.

From this section and the previous one it can be concluded that PS latices are not as ideal a model substrate for adsorption studies as was generally believed some 10 years ago. Nevertheless they constitute still interesting substrates for the investigation of (bio)polymer adsorption, mainly because among the organic colloids available, PS latices are the most studied ones and relatively best characterized ones.

3.3 SILICA AND BOROSILICATE GLASS

3.3.1 Preparation and specific surface area of silica

Pyrogenic (fumed) silicas are prepared from $SiCl_4$ by oxidation in a hydrogen air mixture at high temperatures (1270 K). This results in amorphous spherical SiO_2 particles. Before contact with water hydrophobic ≡Si-O-Si≡ (siloxane) bonds predominate at the surface and only a few ≡Si-OH groups are present. The specific surface areas as found from N_2 or Ar adsorption data are generally in good agreement with the surface found from EM (*Koberstein and Voll, 1970*). Gas adsorption measurements with Ar are preferable above those with N_2 as the adsorbate, because the latter interacts specifically with ≡-Si-OH groups on the surface. The results of *Koberstein and Voll (1970)* indicate that fumed silicas are essentially nonporous.
AEROSIL OX50 (Degussa) used in this study has a specific surface area

of 50 ± 15 m^2g^{-1} as stated by the manufacturer. This value agrees well with that reported by *Sonntag (1980)* for this silica. The surface area obtained from methylene blue adsorption (a_o = 0.55 nm^2) is 3.6 m^2g^{-1} (pH∿6, no added electrolyte) indicating adsorption of MB at ≡Si-O^- sites only, a similar result as was found for MB adsorption on PS latex (section 3.2.2).

3.3.2 Borosilicate glass purification and specific surface area

The glass powder used in this study was a borosilicate glass (732-01) obtained from Sovirel (particles < 60 μm). The composition of the glass as stated by the manufacturer is: 80% SiO_2, 13.00% B_2O_3, 2.25% Al_2O_3, 0.05% Fe_2O_3, 3.50% Na_2O and 1.15% K_2O. Purification of the glass powder was done in essentially the same way as described by *Nyilas (1966)*: First the powder was washed with water twice, then with 6 M HCl at room temperature until no iron could be detected in the washing water by SCN^-. Then the powder was exhaustively washed with destilled water untill the pH of the supernatant was about 6. The powder was then dried at 378 K. The specific surface area of the powder was determined by B.E.T. N_2 adsorption, MB adsorption and electronmicroscopy.

For the glasspowder sample used here the B.E.T. method is not very accurate because of the small (< 1 m^2g^{-1}) value of A_{sp} (see for a discussion *Beurton and Bussiere, 1970*). In addition, the computed value of the surface area is probably also too low, because N_2 has a preference for free OH groups on the surface (*Doremus, 1973*). The B.E.T. N_2 surface area (0.5 ± 0.2 m^2g^{-1}) and the MB adsorption area (A_{sp} = 0.21 m^2g^{-1}, a_o = 0.55 nm^2, pH∿6, no added salt) differ by about a factor of two. The plateau adsorption of MB on the glass from a 50% methanol-water mixture was just half of that in pure water (see fig. 3.6). This points, as was also pre assumed, in the direction of dimer adsorption of MB molecules from water.

Another argument for dimer adsorption of MB from pure water on glass, stems from a comparison with the adsorption of Toluidine blue which is very similar to MB. Toluidine blue shows a colour change from blue to pink-violet upon adsorption on the glass (metachromacy). This is possible when aggregates on the surface exist. A similar effect has been observed for the adsorption of cyanine dyes on silver halides (*Padday, 1970*). Also for the binding of toluidine blue on anionic linear polyelectrolytes the metachromasy is well established (*Horn, 1978*).

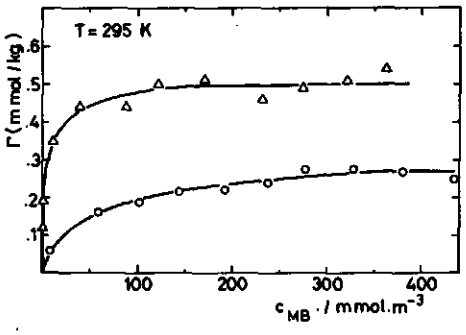


Fig. 3.6 Adsorption of MB on to borosilicate glass (A_{sp}(B.E.T.) = 0.5 $m^2.g^{-1}$). pH (after ads.) = 6.8; no added electrolyte; A/V = 125 $m^2.l^{-1}$. o-o Adsorption from water/methanol (1:1); Δ-Δ Adsorption from water.

Nyilas (1976) found from water vapour adsorption measurements on a soda lime type glass (75% SiO_2, 10% TiO_2/BaO, 5% CaO, 3% B_2O_3 and 2% Na_2O) which had been subjected to the same pretreatment as the glasses used by us that the glass surface was essentially non-porous. This is probably also the case with our glass powders. The conclusion from the above is that also on the glass surface MB molecules adsorb mainly on the charged sites, thus explaining the discrepancy between the N_2 and MB surface area.

The value found for the geometrical surface area following the method described for irregular particles by *Heywood (1970)* is 0.24 m^2g^{-1}, using a calculated shape factor of 11.0. The order of magnitude is the same as the MB surface area. Also the geometrical surface is too low, because a kind of particle enveloppe is measured, thus neglecting small irregularities. Although the MB adsorption isotherm can be determined relatively easily and accurate the calculated MB surface area is very limited because of many complicating factors such as the pH and ionic strength dependence of the adsorption, a conclusion which applies also to the silica and latex adsorbents, and which was also reached by several other authors.

The reasonable agreement between MB adsorption and N_2 adsorption values which exist for the specific surface area of AgI particles in

AgI suspensions as already stated in the previous section (3.3.2), seems to be an exception to the above.
Despite the relative inaccuracy of the B.E.T. N_2 specific surface area of the glass powder, it appears to be the most real value available and therefore this value will be used henceforth.

3.3.3 Determination of the surface charge density by conductometric or potentiometric proton titrations

The charge on the silica-water interface arises from the ionization of surface silanol groups according to the reactions $\equiv Si\text{-}OH \rightleftarrows \equiv Si\text{-}O^- + H^+$ or $\equiv Si\text{-}OH + H^+ \rightleftarrows \equiv Si\text{-}OH_2^+$, the latter reaction is however not observed at pyrogenic silica interfaces (*Abendroth, 1970*).

In the case of glass the ionization of ≡B-OH and ≡Al-OH groups present at the interface plays also a role. The point of zero charge (p.z.c.) of glass is dependent on the amount and nature of basic oxides present at the surface. Because of the pH and ionic strength dependence of the ionization equilibria, the surface charge of the oxides is of course also pH dependent. A surface charge-pH curve can be obtained from a potentiometric proton titration of a sol, in an indifferent electrolyte solution. A description of the method is given for example in the review on the characterisation of aqueous colloids by *James and Parks (1982)* p. 144, 145.

In the present study charge densities are based on the B.E.T. (N_2) surfaces and calculated from $\sigma_o = F(\Gamma_{H+} - \Gamma_{OH-})$ where Γ_{H+} and Γ_{OH-} are the surface excesses of hydrogen and hydroxyl ions per m^2. A point of zero charge can in principle be established by the intersection of σ_o-pH curves determined at varying concentrations indifferent electrolytes. For our system this was found not possible in the case of silica and glass because in the region of the p.z.c. the curves at various ionic strengths are almost horizontal and superimpose over a relatively broad pH traject. Fortunately the σ_o-pH curves are therefore rather unsensitive for the precize value chosen for the p.z.c. (see also *Abendroth (1970)* and *Sonntag (1980)*). The p.z.c. was chosen at pH 3.0 following the reasoning of *Abendroth (1970)*. The same value was used for Aerosil OX50 by *Sonntag (1980)*. For the pyrex glass also a p.z.c. at pH 3.0 was taken.

In the case of AEROSIL OX50 with only H^+ as the counterions, without added salt, accurate potentiometric measurements are difficult to

perform. An estimate of the surface charge can then be obtained from a conductometric titration of the sol with NaOH. The first break in the graph obtained is a measure of the surface charge present at the pH of the first break point.

All conductometric and potentiometric titrations were performed in a wellclosed double walled vessel thermostated at 293.15 ± 0.1 K under a CO_2 free, water vapour-saturated N_2 atmosphere. For experimental details concerning the potentiometric measurements and the apparatus used, see section 6.6.3.2. Experimental details of the conductivity measurements will be given in section 5.2.2. Proton titrations of the suspensions were performed with 0.1 M NaOH or 0.1 M HCl using a Mettler DV10/DV201 automatic burette. Before the start of a titration, CO_2-free N_2, saturated with water vapour of 293.15 K was bubbled through the suspensions for at least 20 min. The titration volume was always 20 cm^3. For the proton-titrations 2.5% (w/v) silica sols and suspensions of 10 g glass powder in 20.0 cm^3 electrolyte solution were used. The suspensions were prepared with CO_2-free conductivity water. With silica 60s after each NaOH addition (1-2 µmol) a constant pH value was reached. In the case of glass powder pH readings were taken when the pH change of the suspension was smaller than 0.01 pH unit/min.

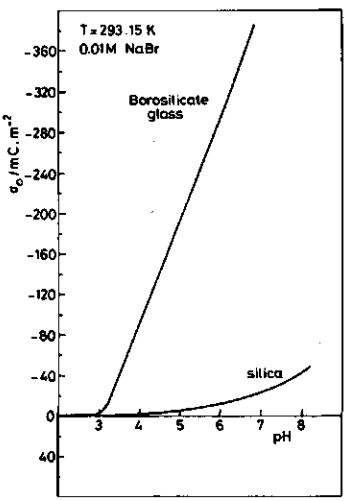


Fig. 3.7 Surface charge pH curves for AEROSIL OX50 silica and Borosilicate glass (Sovirel).

Between pH 4 and 7 this was the case between 5-10 min after the addition of 1-2 μmol NaOH. In fig. 3.7 the surface charge σ_o is plotted as a function of pH in 0.01 M NaBr for the borosilicate glass powder and for the AEROSIL 0X50 used. The results with silica are in reasonable agreement with *Sonntags (1980)* results. He used KCl instead of NaBr as the supporting electrolyte. The behaviour of the borosilicate glass adsorbent is similar to that of the precipitated silica studied by *Tadros and Lyklema (1968)* and *Yates and Healy (1976)*. Also here an unusually high surface charge develops with increasing pH, indicating the existence of a gellayer, only porous for micro-ions. Because this layer is not porous for polyelectrolytes, these will experience a much lower charge upon adsorption.

In fig. 3.8 a conductometric titration of an AEROSIL 0X50 sol without added electrolyte is shown. The surface charge calculated from the first break is 2 mCm^{-2} which is fairly low. So only a very small part of the surface ≡Si-OH groups is dissociated at pH∿6 under these conditions.

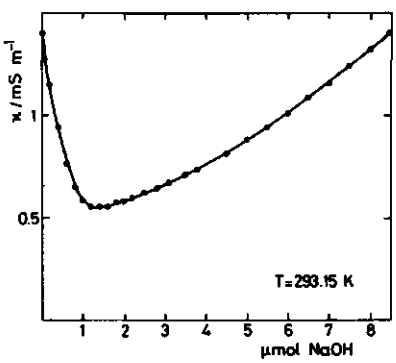


Fig. 3.8 Conductometric titration of AEROSIL 0X50 silica in water, and no added electrolyte.

3.3.4 Some other relevant properties of the silica-water and glass-water interface

When a fresh pyrogenic silica surface is brought into contact with water, siloxane groups on the surface can react with water molecules to form hydrophilic silanol groups:

$$\equiv Si\text{-}O\text{-}Si\equiv + H_2O \rightleftarrows 2\equiv Si\text{-}OH$$

At high temperatures (1270 K) the reverse reaction takes place and the surface becomes more hydrophobic again. Water molecules can also adsorb on silanol groups through hydrogen bonds. On a fully hydroxylated silica surface, two kinds of silanol groups exist: 'free' silanols which do not interact with other OH groups and 'perturbed' or 'bound' silanols which are close enough to other OH groups to form a hydrogen bound. In the infrared spectrum, these two kinds appear as quite different species (*Kiselev, 1975*). It is possible that these two kinds of silanol groups have also a different acidity (*James and Parks, 1982 p. 140*).

The surface concentration of silanol groups is very sensitive to the pretreatment of the silica as will be obvious from the above. For a hydroxylated silica surface in vacuum at 473 K (no physically adsorbed water), *Kiselev (1975, p. 80)* found values between 3.7 and 5.0 ≡Si-OH groups per m^2. The surface silanol concentration for a fully hydroxylated silica in water may be somewhat higher. For a discussion of a few of the more usual methods of identifying and counting surface functional groups, again the review on characterization of aqueous colloids by *James and Parks (1982)* can be recommended. It will be clear that the silica-water interface is heterogeneous with respect to adsorption, a feature that it has in common with PS (latex). The degree of heterogeneity depends on the pretreatment conditons. On a fully hydroxylated and largely hydropilic surface, like AEROSIL OX50 used here, the interactions with a charged adsorbate like polylysine will be mainly of an electrostatic nature. Surface hydrogen bonding can also be part of the adsorption mechanism.

The surface chemistry of glass has recently been reviewed by *Filbert and Hair (1975)*. A section of the review 'Water and interfaces' by *Texter et al. (1978)* deals with the interaction of water with silica and silicate systems. Older reviews are: *Doremus (1973)* and *Deribere-Desgardes and Bre (1967)*. Monographs on the subject have been written for instance by *Holland (1964)* and *Korányi (1963)*. However these reviews deal mainly with the glass-gas interface and pertinent information concerning the borosilicate-glass/water interface is scarce. The properties described above for the silica adsorbent apply also to a large extent to the borosilicate glass. Of the latter substance however the surface chemistry is much more complicated because of the presence of acidic B_2O_3, the amphoteric Al_2O_3 and basic oxides such as Na_2O and K_2O. However the last three are minor components. The surface

composition of a fresh fracture borosilicate surface is probably not much different from the bulk composition. However due to the pretreatment (contact with water and strong acid) the surface content of various oxides differs from the bulk composition. Washings with 6 M HCl as done here with the borosilicate glass, results in a surface which is composed mainly of SiO_2 and B_2O_3, in the form of the ionizable silanol and boranol groups, because of the replacement of alkali and alkaline earth ions by H^+. The original glass backbone structure, is unaffected (*Filbert and Hair, 1975*). Because of the leaching of alkali and basic oxide compounds, a more porous and hydrated surface layer (gellayer) develops, which can have gross effects on adsorption properties. This porosity is not measured with N_2 adsorption, probably because the pores are very small and shrinkage of the gellayer occurs due to the drying of the glasspowder necessary for the gas adsorption measurements. The presence of a more pronounced gellayer than in the case of AEROSIL is evidenced by the charge-pH curves of the adsorbent as already described under section 3.3.3. Also the relatively long pH drift with the borosilicate glass used as compared with pyrogenic silica, points into this direction. Because the gellayer on the borosilicate-water interface is not permeable to large molecules such as polylysine, only the negative charged groups on the 'surface' of this gellayer can directly interact with the $R\text{-}NH_3^+$ groups of polylysine.

Jednačak and Pravdić (1974) measured the electrokinetic potential of glasses in aqueous electrolyte solutions by streaming current measurements. In the experiments the behaviour of quartz and vitreous silica is almost the same. Pyrex glass shows higher negatively potentials than silica, but not as high as one would expect from the difference in surface charge between Pyrex and Silica (see section 3.3.3). This is because most of the charge in the gellayer is compensated for inside this layer. The iso-electric point (i.e.p.) in NaCl solutions is situated at about pH 2.5 for 'pyrex' and at about pH 3.0 for vitreous silica and quartz. Because AEROSIL OX50 is also a pure silica its behaviour will not depart much from that of silica and quartz. The lower i.e.p. for pyrex is expected because of the presence of the constant charge of the $\equiv B\text{-}O^-$ groups.

The properties of glasses as ion exchangers are of particular relevance for the adsorption properties of charged adsorbates like polylysine. The ion exchange properties of glass are reviewed for example by *Doremus (1973)*.

As judged from contact angles of water on various glasses measured by *Moser* (θ = 10-12° for pyrex borosilicate and θ = 7-10° for pure silica (see *Holland (1964)*, p. 359), the pyrex surface seems to be slightly more hydrophobic than the silica surface. However the pretreatment of the glass and the cleaning procedure can have a great influence on the measured contact angle, so that it is questionable if this small difference applies also to the system studied by us.

3.4 SILVER IODIDE AND POLYOXYMETHYLENE CRYSTALS

For comparison reasons some experiments on the adsorption of polylysine were performed with AgI sols and polyoxymethylene crystal suspensions. The AgI sol used was kindy provided by *H.A. van der Schee*. The sol was prepared by the addition in the dark of an $AgNO_3$ solution to a well-stirred KI solution as described by *De Wit (1975)*. The interfacial properties of the essentially hydrophobic AgI surface have been reviewed by *Bijsterbosch and Lyklema (1978)*. A theory of patchwise interfacial hydration of AgI has been put forward by *de Keizer (1981)*.

Polyoxymethylene crystals (POM) are used as an adsorbent for proteins by *Roe (1981)*. The advantage of this adsorbent is that adsorption can take place on a well defined regular flat crystal surface, which bears no charge on the crystal-water interface, at least when no specific adsorption of ions takes place. In this way the electrostatic interactions between adsorbed charged macromolecules can be studied, in principle in a more direct way. The polyoxymethylene crystals used were kindly provided by *M.A. Cohen Stuart*. The crystals were prepared from POM pellets by a recrystallization process from cyclohexanol and subsequent changing the solvent from cyclohexanol via aceton to water in almost the same way as described by *Roe (1981)*.

3.5 SUMMARY

In this chapter a description is given of adsorbent and adsorbate properties relevant for the adsorption of charged adsorbates. Solution properties of polyaminoacids of importance for the adsorption are described.

The determination is described and discussed of the specific surface area and surface charge of the adsorbents most frequently used in this study viz.: PS latex, silica and pyrex glass. Also some other relevant properties of the solid-water interface of these adsorbents are discussed.

3.6 REFERENCES

Abendroth, R.P. (1970). J. Colloid Interface Sci. *34*, 591-596.

Balasubramanian, D. (1974). Biopolymers *13*, 407-410.

Barclay, L. (1970) in 'Surface area determination', Proc. Int. Symp. IUPAC, Everett, D.H. and Ottewill, R.H. eds., Butterworth, London.

Beurton, G. and Bussiere, P. (1970) in 'Surface area determination', Proc. Int. Symp. IUPAC, Everett, D.H. and Ottewill, R.H. eds., Butterworth, London.

Beychok, S. (1967) in 'Poly-α-aminoacids', Fasman, G.D. ed., M. Dekker Inc. New York.

Bijsterbosch, B.H. and Lyklema, J. (1978). Adv. Colloid Interface Sci. *9*, 147-251.

Deribere-Desgardes, M.L. and Bre, M.M. (1967). Symp. of the Union Scientific Continentale du Verre. 'The surface of glass and its modern treatments', Luxemburg.

Doty, P. and Gratzer, B. (1961) in 'Polyaminoacids, polypeptides and proteins', Stahmann, M.A. ed. Univ. Winconsin Press, Madison.

Doremus, R.H. (1973). 'Glass Science'. Wiley-Interscience New York-London.

Fasman, G.D. (1967) in 'poly-α-aminoacids', Fasman, G.D. ed., M. Dekker, New York.

Filbert, A.M. and Hair, M.L. (1975). Adv. in Corrosion Sci.: Technol. *5*, 1-54. Fontana, M.G. and Stachle, R.W. eds., Plenum Press New York-London.

Flory, P.J. (1969). 'Statistical mechanics of chain molecules', Interscience Publishers, Wiley & Sons, New York.

Furusawa, K.; Norde, W. and Lyklema, J. (1972). Kolloid-Z.Z. Polym. *250*, 908-909.

Goossens, J.W.S. and Zembrod, A. (1979). Colloid Polym. Sci. *257*, 437-438.

Greenfield, N. and Fasman, G.D. (1969). Biochemistry *8*, 4108-4115.

Grourke, M.J. and Gibbs, J.H. (1971). Biopolymers *10*, 795-808.

Hermans, J. (Jr) (1966). J. Phys. Chem. *70*, 510-515.

Heywood, H. (1970) in 'Surface Area Determination', Proc. Int. Symp. IUPAC, Everett, D.H. and Ottewill, R.H., eds., Butterworth London.

Hearn, J.; Wilkinson, M.C. and Goodall, A.R. (1981). Adv. Colloid Interface Sci. *14*, 173-236.

Hoven, T. van den (1984). Doctoral thesis Agricultural University Wageningen. In press.

Hul, H.J. van den and Vanderhoff, J.W. (1968). J. Colloid Interface Sci. *28*, 336-337.

Holland, L. (1964). 'The properties of glass surfaces', Chapman and Hall London.

Horn, D. (1978). Progr. Colloid Polym. Sci. *65*, 251-264.

Idelson, M. and Blout, E.R. (1958). J. Amer. Chem. Soc. *80*, 4631-4634.

James, R.O. and Parks, G.A. (1982) in 'Surface and Colloid Sci. *12*, 119-216. Matijevíc, E. ed.

Jednačak, J.; Pravdić, V. and Haller, W. (1974). J. Colloid Interface Sci. *49*, 16-23.

Kamel, A.A.M. (1981). Ph.D. thesis. LeHigh Uiversity Bethlehem, Pennsylvania.

Kasper, D.A. (1971). Ph.D. thesis. California Institute of Technology Pasadena, California.

Keizer, A. de (1981). Doctoral thesis Agricultural University Wageningen.

Kiselev, A.V.; Lygin, V.I. (1975). 'Infrared Spectra of Surface Compounds', Halsted Press, Wiley and Sons, New York, Toronto.

Koberstein, E. and Voll, M. (1970), Z. Phys. Chem., Neue Folge *71*, 275.

Koopal, L.K. (1978). Doctoral thesis Agricultural University Wageningen.

Korányi, D. (1963). 'Surface properties of silicate glasses', Akadémiai Kiadó, Budapest.

McGarvill, W.T. and Fitch, R.M. (1978). J. Colloid Interface Sci. *67*, 204-212.

Nagasawa, M. (1970). Pure Appl. Chem. *26*, 519-536.

Norde, W. (1976). Doctoral thesis Agricultural University Wageningen.

Norde, W. and Lyklema, J. (1978). J. Colloid Interface Sci. *66*, 257-266.

Nyilas, E.; Chiu, T-H. and Lederman, D.M. (1976) in 'Recent Adv. Colloid Interface Sci.' *3*. Kerker, M., ed. Acad. Press New York.

Padday, J.F. (1970) in 'Surface Area Determination', Proc Int. Symp. IUPAK., Everett, D.H. and Ottewill, R.H., eds. Butterworth London.

Pauling, L.; Corey, R.B. and Branson, H.R. (1951). Proc. Nat. Acad. Sci. U.S. *37*, 205-211.

Painter, C.P. and Koenig, J.L. (1976). Biopolymers *15*, 229-240.

Rippon, W.B. and Walton, A.G. (1971). Biopolymers *10*, 1207-1212.

Roe, R.J.; Shastri, R. and Wille, W. (1981). J. Colloid Interface Sci. *84*, 346-354.

Sigma Chemical Co. (1980). Fine chemicals catalog.

Schee, H.A. van der (1981) in 'The effect of polymers on dispersion properties', Tadros, Th.F. ed. Academic Press London.

Sonntag, H. and Kolesnikova, R.S. (1980). Z. Phys. Chem. *261*, 226-232.
Tadros, Th.F. and Lyklema, J. (1968). J. Electroanal. Chem. *17*, 267-275.
Tiffany, M.L. and Krimm, S. (1969). Biopolymers *8*, 347-359.
Tiffany, M.L. and Krimm, S. (1972). Biopolymers *11*, 2309-2316.
Texter, J.; Klier, K. and Zettlemoyer, A.C. (1978). Progr. Surface Membrane Sci. *12*, 327-391.
Wit, J.N. de (1975). Doctoral thesis Agricultural University Wageningen.
Yaron, A. and Berger, A. (1963) Biochim. Biophys. Acta *69*, 397-399.
Yates, D.E. and Healy, T.W. (1976). J. Colloid Interface Sci. *55*, 9-19.
Vanderhoff, J.W.; Hul, H.J. van den; Tausk, R.J.M. and Overbeek, J.Th.G. (1970) in 'Clean Surfaces'. Goldfinger, ed. M. Dekker New York.
Zimmerman, S.S. and Mandelkern, L. (1975). Biopolymers *14*, 567-581.

4 ADSORPTION OF THE HIGHLY CHARGED POLYELECTROLYTE POLYLYSINE ON DIFFERENT SUBSTRATES

4.1 INTRODUCTION

The main subject of this chapter is the adsorption behaviour of poly-L-lysine at maximal charge density in solution (pH<8) and the influence of the nature of the substrate on this adsorption.

A special feature of poly-L-lysine as a polyelectrolytic adsorbate is the chain charge density dependent conformation of these macromolecules in aqueous electrolyte solutions. At low chain charge density (pH>10) the polyaminoacid is in the α-helix conformation, thus resembling a structure which also occurs in most protein molecules. However at high charge density (pH<8) the molecules are, depending on the electrolyte concentration, more or less flexible polyelectrolytes. Then they are suitable model substances to investigate the influence of molecular mass, ionic strength, surface charge, and nature of the adsorbent on polyelectrolyte adsorption.

On the other hand, the unravelling of the adsorption behaviour of the polylysine helix and the role of the helix-coil transition may be of fundamental importance in the understanding of the behaviour of biomacromolecules with secondary and tertiary structure at solid-liquid interfaces. This will be the subject matter of chapter 6.

The adsorption behaviour of both flexible and rigid biological macromolecules, as well as flexible synthetic polyelectrolytes is of importance in a number of (bio)medical, biological and (bio)technological processes. Some examples are: The use of synthetic polyelectrolytes as polymeric flocculants for particles oppositely charged to the polyelectrolyte in water purification and the adsorption of salivary polypeptides on hard tooth tissue in relation to the development of tooth caries. Polyelectrolyte adsorption does also play a role in the blood-clotting process at interfaces. More examples are given by *Hesselink (1983)*.

4.2 EXPERIMENTAL

4.2.1 Materials

poly-L-lysine.HBr, poly-DL-lysine.HBr, poly-L-ornithine.HBr and poly-L-histidine were obtained from the Sigma chemical Co and used without

further purification. Viscosity average molecular masses of the polyaminoacids as stated by the manufacturer were used in this study (see also 3.1). All other chemicals used were of analytical grade. The water used was distilled once or conductivity water obtained from a millipore super Q water purification apparatus. The preparation of the polystyrene latices was essentially the same as described by *Furusawa et al. (1972)*. Purification of the latices was done by steamstripping and ion exchange of the latices with extensively purified Dowex ion exchange resins (BIORAD). For details see (section 3.2).

The glasspowder used was a borosilicate glass (732-01) obtained from Sovirel and purified in the same way as described by *Nyilas (1976)*. The B.E.T. N_2 surface area amounts to 0.5 ± 0.2 m^2g^{-1}.

The silica used was AEROSIL OX50 (Degussa), with a specific surface area of 50 ± 15 m^2g^{-1} as stated by the manufacturer. The silica was used without further purification. Dispersions of the silica powder in conductivity water were prepared by suspending silica that was dried for two hours at 413 K in water and ultrasonication (25 kHz) of the suspension under cooling (~287 K) for one hour.

The AgI sols and polyoxymethylene crystal suspensions used in some of the experiments were gifts of H.A. v.d. Schee and M. Cohen Stuart respectively from our laboratory. Some characteristics of these suspensions are given in section 3.4.

4.2.2 Determination of the polyaminoacid concentration

The concentration of the positively charged polyaminoacids in aqueous solution was determined by a complex titration with the potassium salt of polyvinylsulphate, using toluidine blue as the indicator (*Terayama, 1952; Horn, 1978*). In each series of polyaminoacid determinations standard samples of known concentration were included for the construction of a calibration curve. Addings of polyvinylsulphate were done with a Mettler DV10/DV 201/(1 cm^3) automatic burette.

4.2.3 Adsorption measurements

Adsorption isotherm measurements were carried out by adding aliquots of the sol to 4.0 cm^3 polyaminoacid solution (in the case of glasspowder 5.0 cm^3 PA solution was added to 2.0 g glasspowder) in 10 cm^3 polycarbonate centrifuge tubes with polyethylene caps. For pH values higher than 8 polyethylene tubes were used. Prior to mixing, both solutions

were brought to the same pH and ionic strength, without using buffers. The tubes were rotated end over end for 16 hours, in a thermostatted room (295 K), to ensure equilibrium. When PS latex without added salt (pH∿6) was added to a polyaminoacid solution with such a pH and ionic strength (obtained by adding known amounts of 0.1 M NaOH or HCl, NaBr solution and water to a PL stock solution) that after mixing the desired ionic strength and pH value was reached, no different adsorbed amount of PL was found with respect to the former method of sample preparation. This shows that the adsorbed amount found was independent of the way in which the final state was reached.

The amount of PA-acid adsorbed was determined from the depletion of the solution. To this end, the samples were centrifuged for 20-30 min at 18.000 r.p.m. (37.000 g at r = 102 mm) in a Beckman JA-21 centrifuge with JA-21 rotor. The PA concentration in the clear supernatant was measured as described under 4.2.2. The measurements were performed at room-temperature (295 ± 2 K). pH measurements were done with an electrofact 36200 pH meter or an Anker Smith A161 digital pH meter. Combined glass - Ag/AgCl electrodes from Electrofact (7GR131) or Schott (N58) were used.

4.2.4 Conductometric and potentiometric titrations

All conductometric and potentiometric titrations for particle surface charge determinations were performed in a well closed double walled vessel, thermostatted at 293.15 ± 0.05 K under a CO_2-free water vapour saturated N_2 atmosphere. An Ankersmith A161 digital pH meter and Schott N58 or N59 combined glass-Ag/AgCl or glass-calomel electrodes were used for the measurements. The pH meter was standardized with titrisol (Merck) buffers pH 7.00; 4.00 and 9.00 before each titration. Conductometric titrations of PS latex (H^+ form) or silica were performed with a Retch conductivity meter operating at 4 kHz. Conductivity cells with cell constants of 10.40 m^{-1} or 71.94 m^{-1} (platinum black) were used. Addings of calibrated 0.1 M NaOH or 0.1 M HCl (Titrisol) were done with a Mettler DV10/DV201 or Methrohm 655 Dosimat automatic micro burette.

Surface charge-pH curves are obtained from the potentiometric proton-titration curves of silica and borosilicate glass powder dispersions, and the corresponding blank titrations in the usual way, defining $\sigma_o = F(\Gamma_{H^+} - \Gamma_{OH^-})$ where $(\Gamma_{H^+} - \Gamma_{OH^-})$ is the adsorption excess of hydrogen over hydroxyl ions in equivalents per m^2 (N_2 surface). See for example

Abendroth (1970), James and Parks (1982), Ardizzone et al. (1982). Some more experimental details concerning the silica and glasspowder dispersion titrations have been given in section 3.3.3. The surface/volume ratio is 1250 $m^2.l^{-1}$ for the AEROSIL OX50 titrations and 250 m^2l^{-1} for the glasspowder titrations.

4.2.5 Stability measurements

Stability measurements of bare and poly-L-lysine covered polystyrene and AgI sol particles against low molecular mass electrolyte were performed by a static method. Five cm^3 sol (with or without PL) was mixed rapidly with 5 cm^3 electrolyte solution and the mixture was kept at 298.15 K in a thermostatted water bath. This was repeated for different electrolyte concentrations. After a total equilibration time of 18 hours, the OD_{10mm}^{540nm} of the top 2 cm^3 of the dispersions was measured using a Beckman model 3600 spectrophotometer. Optical data are expressed as the residual turbidity τ_r, which is the optical density of the suspension τ, obtained in the presence of electrolyte, relative to that measured for an identical system in the absence of electrolyte and polyelectrolyte τ_o .: $\tau_r = \tau/\tau_o$.
Thus τ_r equals 1.0 for stable suspensions and control systems. The obtained relative turbidity values were plotted against the electrolyte concentration to judge the stability of the suspensions.

Stock sols with added poly-L-lysine were prepared as follows: To a diluted latex (~0.01% w/v) or AgI sol (pI = 4)(~0.01% (w/v)) a PL-L solution of the desired concentration in water was added rapidly by means of a pipetman dispensing pipet. The total pipetted volume was such, that rapid mixing due to the liquid jet was achieved. The obtained stable sol was rotated end over end for at least two hours at 295 K to ensure adsorption equilibrium. Blank sols for stability measurements were prepared in the same way by pipetting conductivity water to the diluted sol instead of PL solution. The particle concentration of the obtained stable test sol, after electrolyte addition, was always such that the OD_{10mm}^{540nm} was between .3 and .8.

4.3 RESULTS AND DISCUSSION

4.3.1 Comparison of the specific surface areas of the adsorbents

When one wants to compare the adsorption saturation values of an adsorbate on different adsorbents, the specific surface area of these ad-

sorbents must be known. Also other characteristics of the surfaces such as the surface charge density and the distribution of adsorption sites in the case of heterogeneous surfaces, should be known, to allow a meaningfull comparison. From a practical point of view the last demand is almost not realizable.

There is always some intrinsic uncertainty on the value to be assigned to the specific surface area. The surface area as 'seen' by the polylysine molecules is not necessarely the same as that determined with, for example N_2- or dye adsorption. Hence some reserve is always needed in making comparisons of absolute adsorption data. This particular difficulty is not encountered if shapes of adsorption isotherms are compared, where the adsorbed amount is expressed as fractional coverage.

Consider for example the negatively charged polystyrene surface as an adsorbent for the positively charged polylysine. The negatively charged sulphate groups will act as strong adsorption sites for the positively charged aminogroups. The space between the sulphate groups consisting of benzene and $-CH_2$ groups of the polystyrene surface, has probably a much lower affinity for the polylysine chain. In the case of the adsorption of small molecules adsorbing on the negatively charged sulphate groups only, one can define the specific surface area for this compound as the sum of the surfaces of all sulphate groups per gram adsorbent. The obtained plateau adsorption is then 'monolayer' adsorption per definition. In the case of a positively charged macromolecule this definition has little meaning because of the possibility of bridges between negatively charged surface sites. Such bridges can be polymer loops but also trains. In other words, the positive units used to count the number of negative surface sites are no longer independent. For this reason, I chose the geometrical or N_2 gas adsorption (which are not much different for the adsorbent used here) for conversion of the adsorbed amount into $mg.m^{-2}$. The N_2 gas adsorption area approaches most probably the physical surface area in our cases, i.e. it is the sum of the area of all strong and weak PL adsorption sites per gram adsorbent.

When the distribution of strong adsorption sites is homogeneous over the surface, the obtained plateau adsorption values are a measure off the fraction of the 'total' surface area covered by polyelectrolyte.

In tabel 4.1 some relevant properties of the adsorbents used in this study are given. A discussion of the values found has been given in section 3.2 and 3.3. For the adsorption measurements on silica the value of 50 m^2g^{-1} was used arbitrarely for the specific surface area in most cases.

Table 4.1 Some relevant properties of the adsorbents used in this study

Material	Nature of charge determining groups	A_{sp} ($m^2.g^{-1}$) N_2 ads.	A_{sp} ($m^2.g^{-1}$) EM (geo.)	A_{sp}($m^2.g^{-1}$) MB ads. ($pH_{ads} \sim 6$)	Surface charge ($mC.m^{-2}$)	
PS latex						
M_1*	$-OSO_3^-$	-	9.3	-	-74	pH>3; c_s<0.1M
M_2	"	-	10.4	-	-60	" c_s<0.01M
M_3	"	-	9.2	4.0	-52	" "
M_4	"	-	10.0	-	-40	" "
M_5	"	-	9.2	1.7	-39	" "
L	"	-	11.7	-	-11	" "
P	$-^+N(CH_3)_2C(CH_3)_2$	-	14.8		+	pH<9
Glasspowder						
B_1	$\equiv B-O^-$; $\equiv Si-O^-$	0.4±0.2		0.16	-292	pH=6; 0.1M NaBr
B_2	"	0.5±0.2	0.24	0.21		
Silica						
AEROSIL	$\equiv Si-O^-$	50±15**	50±15**	3.6	-22	pH=6; 0.1M NaBr
OX50		60.3±1.5****				
AgI	Adsorbed		-	9***	~-40	pI=5; 0.1M KNO_3
sol	I^- and/or Ag^+					
prec.	depending on pAg		-	0.19***	"	pI=5; 0.1M KNO_3

* Latex was brought to 0.1 M NaBr before ion exchange

** Degussa; *** van der Schee (1982); **** our laboratory.

4.3.2 Adsorption time

The amount of PL-L (DP = 240) adsorbed from 0.1 M NaBr (pH = 6) on PS latex particles M_3 was measured after 0.5; 1.3; 1.6 and 30 hours of contact time. The latex concentration was the same as used for determining adsorption isotherms. At each mixing time several starting concentrations of PL were used, all resulting in the same adsorption value at all contact times. The equilibrium concentrations varied from 20-200 gm^{-3}. The results are plotted in fig. 4.1.

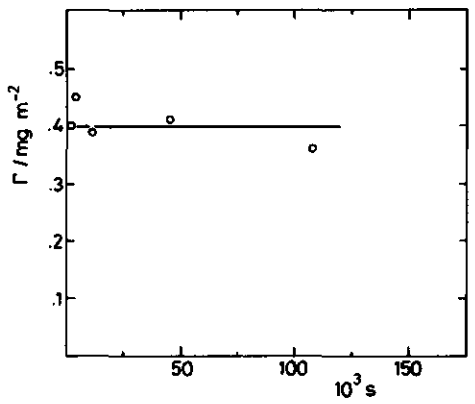


Fig. 4.1 Adsorbed mass of PL.HBr-L (DP 240) as a function of contact time in 0.1 M NaBr; pH = 6; T = 295 K; PS latex M_3.

It can be seen that within experimental error the adsorbed amount Γ is constant in the time range studied. A similar result was obtained for the adsorption of PL-L (DP 1923) from 0.01 M NaBr on borosilicate glass (pH ∿ 6). Also *van der Schee (1982)* found the same result for the adsorption of PL-L on AgI. Investigations of contact times shorter than 30 min is not possible with our technique, because the centrifugation time is then of the same order of magnitude as the contact time. Another technique for separating adsorbate and adsorbent is then required.

The time scale observed here for polyelectrolyte adsorption process (faster than 30 min.) is often observed for the adsorption of polyelectrolytes, oppositely charged to the surface (*Rawls et al., 1982; Williams et al., 1982, Horn and Melzer, 1976; Eggert, 1976; Lindquist, 1975*). The strong attractive electrostatic interaction between polymer

and colloidal particles was hold responsible for this by *Eggert (1976)*. However this argument is only applicable up to the point of charge neutralization. The increase of the adsorbed amount which takes place beyond this point (dependent on the electrolyte concentration), should be a slower process. *Corry (1978)* found for the adsorption of PL-L on latex, equilibrium times of 5 min as judged by changes in electrophoretic mobility with time after addition of PL.
The polyelectrolyte adsorption process is faster than generally observed for uncharged polymer adsorption. For such systems it is often found that the adsorbed amount still increases during the first ten hours of contact time or even longer (*Cohen Stuart, 1980; Koopal, 1978*). The cause of this relatively long adsorption time is thought to be reconformation processes of the polymer at the interface and in the case of heterodispers polymers, the exchange of small molecules adsorbed at the interfaces against bigger ones. This gives rise to an increase in the adsorbed amount (*Cohen Stuart et al., 1980*).

Of course reconformation and polymer heterogeneity also play a role in the polyelectrolyte adsorption process. The long equilibrium times for the adsorption of poly(2 sulphoethylmethacrylate) (M_v/M_n = 1.2) on polyethylene powder at 0.1 M NaCl found bij *Greene (1971)* can be attributed to this effect. For PL-L used here the M_w/M_n ratio is probably closer to 1. Moreover the molecular mass dependence of the adsorption is also very small above $\bar{M}_v$ 30.000, so that exchange of short chains against longer ones cannot give a measurable increase in the adsorbed amount and hence this effect has no effect on the equilibrium time. This absence of any detectable polydispersity effect can be responsible for the relatively short equilibrium times too.

Although the adsorbed amount of PL remains constant after 30 min of contact time, the composition of the adsorbed layer may still not yet have its equilibrium composition. For this reason and for practical convenience an adsorption time of 16 hours was chosen in most of the experiments.

4.3.3 Effect of molecular mass

In fig. 4.2 the molecular mass dependence of PL-L adsorption on latex M_2 in 0.1 M NaBr at pH 6 is shown. The starting concentration of PL.HBr was such in these experiments that plateau adsorption was assured. The adsorption of PL increases with M up to $\tilde{\sim}$ 50.000 (DP 240) after which the molecular mass dependence is only slight. Such an behaviour is theo-

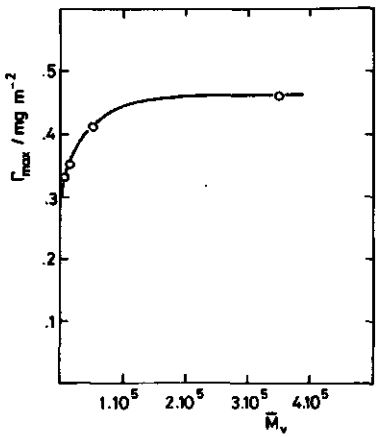


Fig. 4.2 Plateau adsorption of poly-L-lysine on PS particles (latex M_2 σ_o = -60 mC.m^{-2}) in 0.1 M NaBr at pH 6, as a function of the PL molecular mass.

retically expected for uncharged polymers, adsorbing from a good solvent. Because of the long range electrostatic interactions between PL segments the meaning of the χ parameter is not obvious in this case. In the absence of added electrolyte ($I<10^{-4}$) there is no measurable molecular mass difference at all between DP 19 and 240, indicating flat adsorption. With respect to the molecular mass dependence, the behaviour of PL.HBr, adsorbed on glass and AgI, is qualitatively the same as described here for the PS surface. In the case of the glass powder this shows that the glass surface is not significantly porous for PL because otherwise PL, with a low degree of polymerization, should give higher amounts of adsorption at higher degrees of polymerization. Such an effect of pore size was observed indeed by *Horn and Melzer (1978)* for the adsorption of polyethyleneimines on cellulose fibers.

The molecular mass dependence in the polylysine-polystyrene system at higher salt concentration is mainly caused by the decreased electrostatic repulsion between the chain segments, so that more loops and tails can develop. At very high ionic strength the lower solvent quality of the electrolyte solution can also contribute to a higher Γ. This effect is stronger for PL of high DP. These results are in qualitative agreement with the predictions of the polyelectrolyte adsorption theory of van der Schee (*Bonekamp et al., 1983*). Experimental results of *van der Schee (1983)* for the PL-AgI and *Eggert (1976)* for the poly

(1,2-dimethyl-5-vinyl(pyridinium)bromide-polystyrene latex system show the same trends as observed here for the PL-PS.latex system.

Because of the slight molecular mass dependence of the PL adsorption on polystyrene and the appearance of very sharp adsorption isotherms, an influence of the adsorbent surface to volume ratio on the isotherms, due to the polymer heterodispersity (*Cohen Stuart et al., 1980*), is not expected at low salt concentrations and low pH. There were no differences indeed between isotherms of PL-L adsorbed on PS and glass measured with A/V ratios between 80 and 400 $m^2.l^{-1}$ for the glasspowder adsorbent and 48-278 m^2l^{-1} for PS latex. The salt concentration was 0.01 M NaBr. Accurate measurements at low PL equilibrium concentrations showed however that at higher ionic strength (0.1 M; pH 6) the adsorption isotherms of PL on PS become somewhat more rounded, indicating an effect of the PL heterodispersity, which was even more pronounced at 0.5 and 1.0 M NaBr. However at these higher salt concentrations the PL concentration determination is less accurate, which can also contribute to the observed roundedness of the isotherms.

4.3.4 Adsorption isotherms

In fig. 4.3 (a,b,c) characteristic adsorption isotherms of highly charged polylysine on different adsorbents are plotted. The adsorbed amount is expressed in $mg.m^{-2}$ PL.HBr in all cases using the N_2 or geometric specific surface areas given in table 4.1. In all cases the isotherms have a very high affinity character and a well-defined plateau value. Of course this does not mean that the affinity of PL for the various surfaces is not very different, but only that any difference is not measurable. In the case of PL adsorption on PS some isotherms were measured with equilibrium concentrations PL up to 900 $g.m^{-3}$. No raise in adsorption after reaching the plateau value could be observed. As said, at low salt concentrations ($<10^{-3}$M) the plateau values are independent of molar mass, indicating adsorption in a flat conformation, i.e. with trains only. There is no measurable difference in plateau value between PL-L and PL-DL between all adsorbents investigated. This is expected because both polymers are known to be in a coil conformation at pH values lower than 9 (*Applequist and Doty, 1962*). The non-electrostatic persistence length of PL-DL ($l_p \simeq 0.8$ nm) is about twice as low as the value for PL-L ($l_p \simeq 2$ nm) (e.g. *Flory, 1969; Brant and Flory, 1965*).

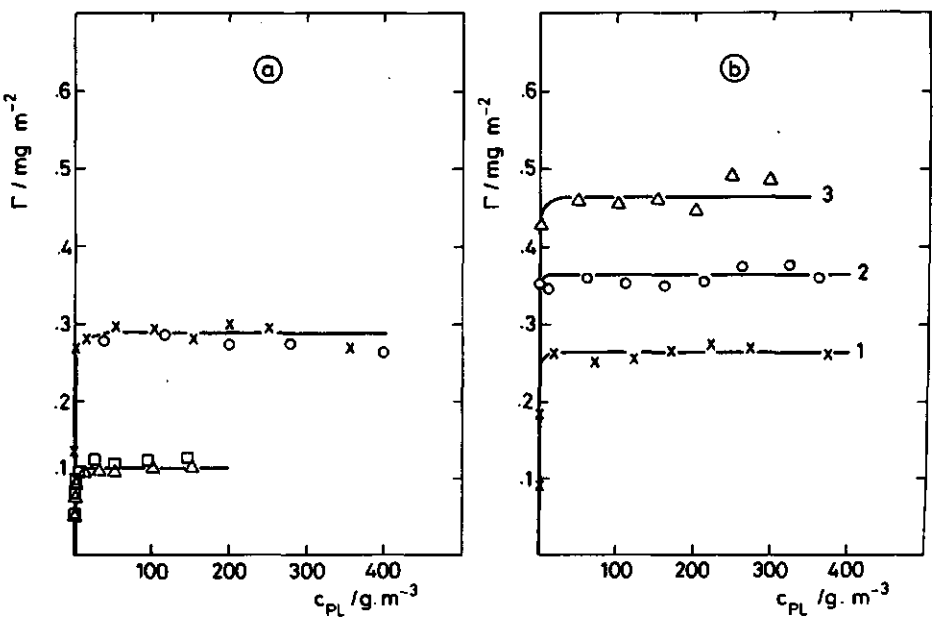


Fig. 4.3 a. Adsorption of polylysines on silica and borosilicate glass. T = 293 K.
x-x PL-L (DP 240)/glass; 0.01 M NaBr, pH 5.9
o-o PL-DL (DP 240)/glass; 0.01 M NaBr, pH 5.9
Δ-Δ PL-L (DP 190)/AEROSIL; 0.01 M NaBr, pH 4.7
□-□ PL-L (DP 190)/AEROSIL; 0.01 M NaSCN, pH 5.0

b. Adsorption of poly-L-lysine (DP 1683) on PS (latex M_2). T = 293 K.
x-x 10^{-3}M NaBr, pH 6; o-o 10^{-2}M NaBr. pH 6; Δ-Δ 10^{-1}M NaBr pH = 6.

In principle this could lead to different adsorption values for PL-L and PL-DL at salt concentrations above 0.1 M. Above this concentration the total persistence length L_T is mainly determined by the non-electrostatic concentration l_p. At low salt concentrations PL-L and PL-DL are about equally flexible, because the calculated electrostatic persistence length ($l_e \simeq 36$ nm, 10^{-3} M NaBr; $l_e \simeq 0.4$ nm, 10^{-1} M NaBr), which is dominant at low salt concentration, is identical for PL-L and PL-DL. No differences in Γ due to differences in L_T are expected then. Monolayer coverages for the fully extended PL-L and the most compact conformation (pH<9), obtained from Stuart models, amount 0.67 and 1.0 mg m^{-2} PL.HBr (0.41 and 0.61 mg.m^{-2} PL (without Br^-)) respectively (*van der Schee and Lyklema, 1981*). Because of entropical reasons an inter-

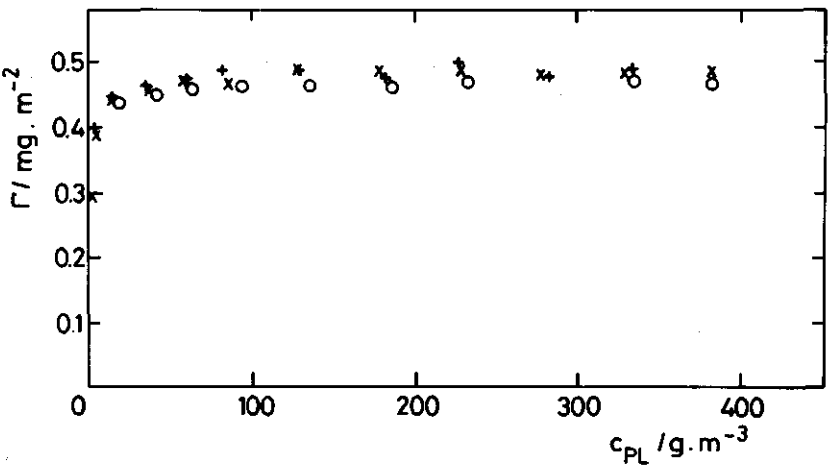


Fig. 4.3c Adsorption of polylysines on silver iodide.
x-x PL-L (DP 300); o-o PL-L (DP 2000); +-+ PL-DL (DP 250).
σ_o = -10 mC.m^{-2}, electrolyte 10^{-2} M HNO_3, T = 293 K.
(after van der Schee (1984), van der Schee and Lyklema (1982).

mediate situation seems the most probable. Comparison with the experimental values in 10^{-3} M electrolyte solution suggests that the molecule adsorbs in a rather extended fashion i.e. it has a large contact area with the adsorbent used. Implicitly it is assumed then that the surface charge is distributed homogeneous over the surface. The assumption of a homogeneous surface charge distribution is better for AgI, where the surface charge has a more smeared out character, than for silica, glass and certainly polystyrene were discrete charged groups exist.

The adsorbed amount as such is indicative of the thickness of the adsorbate layer only, when one is sure that the distribution of adsorption sites (i.e. patches with high adsorption free energy) is homogeneous and the density of patches is known (see also 4.3.1). In this case the conclusion of flat adsorption of PL at low salt concentration, obtained from the low value of the adsorbed amount remains valid. A homogeneous distribution of adsorption sites is very likely for AgI and the SiO_2 adsorbent. In the case of PS latex it is not sure wether the $-OSO_3^-$ groups are homogeneously distributed over the surface. A homogeneous distribution seems probable in this case considering the emulsion polymerization mechanism of styrene. However *van der Put (1981)* suggested the presence of a patchwise distribution of $-OSO_3^-$

groups on the PS surface from electrokinetic measurements.

Differences in the absolute values of the adsorbed amounts of PL between different substrates have only a meaning when greater than, say 40-50%, because of the large uncertainties in the specific surface areas of the adsorbents. For the glass powder adsorbent the uncertainties are probably even greater because of the relatively great error in A_{sp}.

The effect of the hydrophobicity of the adsorbent on the adsorbed amount, can only be directly studied by comparing adsorption data on adsorbents, which differ in hydrophobicity, at constant surface charge, which is experimentally very difficult to achieve. When we take the adsorption of PL-L on PS latex from 10^{-2} M NaBr pH 6 as a reference value (0.37 $mg.m^{-2}$), then adsorption values under the same conditions for the other adsorbents higher than 0.56 or lower than 0.19 $mg.m^{-2}$ may be considered to deviate significantly (see fig. 4.3).

Because of the above, nothing definite can be concluded from fig. 4.3 about the influence of differences in non-electrostatic contributions, to the adsorbed amount between the adsorbents used. In no case PL forms a complete monolayer. The dominant role of electrostatic interactions (repulsive and attractive) in these systems must be the main cause for this. Such a conclusion was also reached by several other authors (e.g. *Cafe and Robb, 1982; Williams et al., 1982*). For example *Williams et al. (1982)* concluded that the adsorption of negatively charged carboxymethyl cellulose on negatively charged $BaSO_4$ is mainly limited by the strong repulsion between the segments of the adsorbed phase.

The adsorbed amount PL on PS and AgI expressed in $mC.m^{-2}$ is much higher than the surface charge. As the PL molecules are very likely to be adsorbed flat, the adsorption must be superequivalent too. That this was the case indeed was shown by *van der Schee and Lyklema (1982)* for the PL-AgI system by means of electrophoretic mobility measurements. The same was shown by us for the PL-PS latex system also from electrophoretic mobility measurements and from electrokinetic measurements on PS plugs with adsorbed PL. When the surface charge is thought to be smeared out, superequivalent adsorption is only possible when also other attractive interactions contribute to the adsorption energy per segment χ_s.

In the case of PS hydrophobic bonding between the $-(CH_2)_4-$groups of the lysine side chain and the phenyl groups of the PS particles can be responsible for this. With AgI the same argument applies, but also complex formation between Ag and $-NH_2$ is a possibility. The occurrence

of superequivalent adsorption is not certain in the case of silica and glass. The adsorption of PL on these substrates is probably not completely of an electrostatic nature, because no complete desorption occurs at high ionic strength as I will show later on. Hydrogen bonds between the peptide groups of PL and the surface silanol groups may contribute to χ_s in these cases.

When there is a non-electrostatic contribution to the adsorption energy some adsorption at the uncharged surfaces is expected, at least at higher salt concentrations. With the PL-AgI system this is indeed the case (*van der Schee*, personal communication). In the case of silica no PL adsorption at the p.z.c. could be detected. No direct measurements are available for the adsorption of PL on uncharged PS latex particles (see section 4.3.5). No adsorption of PL could be detected at the uncharged polyoxymethylene surface, at least below or at 0.1 M NaBr. To obtain information about the electrostatic and non-electrostatic contributions, plots of the adsorbed amount versus ionic strength and surface charge for the various adsorbents, are more imformative than the adsorption isotherms in fig. 4.3 (a,b,c). This will be the subject of sections 4.3.5 and 4.3.6.

There was no detectable desorption of PL from PS upon dilution. The same feature was found for the AgI system by *van der Schee (1984)* and for example by *Williams et al. (1982)* for the carboxymethylcellulose-bariumsulphate system. The non-desorbability of the macromolecular adsorbate is not due to a real irreversibility (i.e. the adsorbate is not a frozen non-equilibrium state) but to the extreme dilutions that are required to remove higher M compound from the surface (*Cohen Stuart et al., 1980*). Some indications were obtained, that PL adsorbed on glass can be displaced from the surface by MB.

For comparison purposes isotherms of poly-L-histidine (DP 115), poly-L-ornithine (DP 103) and PL-L (DP 62) were simultaneously measured at 293 K and 0.1 M NaBr with PS (latex M_2) as the substrate at pH = 3 (all polyaminoacids are completely charged). The plateau values found were respectively 2.1 $\mu mol_r.m^{-2}$, 1.8 $\mu mol_r.m^{-2}$ and 1.8 $\mu mol_r.m^{-2}$ and not significantly apart. This supports our conclusion of the dominating importance of electrostatic interactions over possible effects of the different side chain structure on the adsorbed amount (i.e. differences in χ_s). The poly-L-histidine isotherm was more rounded than the other two. This is indicative for a broader molecular mass distribution for this sample.

4.3.5 Influence of the surface charge density

From the point of view of the polyelectrolyte adsorption theory of *van der Schee (1982, 1984)* it is important to know the measured plateau adsorption, not only as a function of the pH or pAg in the case of silica and AgI respectively, but also as a function of the surface charge. This is because the numerical calculations with the *v.d. Schee (1984)* theory are for the moment expressed as a function of σ_o only. For PS this is no problem since the surface charge is constant, but on SiO_2 and AgI, σ_o changes when PL adsorbs. However the experimental trends are roughly the same whether the plateau adsorption is considered as a function of σ_o or as a function of the pH or pAg.

As said the polystyrene-water interface, negatively charged due to the presence of covalently bound $-OSO_3^-$ groups in the interface, is in principle a so-called constant-charge surface. This means that the surface charge σ_o is not altered due to changes in salt concentration, electrical double layer overlap and specific adsorption. This is clearly not the case with AgI and silica, where at constant pAg and pH respectively, the surface charge increases with increasing salt concentration. The charge-pAg curves of AgI in the presence of polylysine are shifted to higher σ_o values and a lower p.z.c. due to the adsorption of PL (*van der Schee and Lyklema, 1982*). The shift to higher σ_o values is much more pronounced in the case of silica with adsorbed PL (plateau adsorption at each pH value). On AgI the adsorbed positively charged PL, promotes the adsorption of I^- ions and σ_o becomes more negative. By the same token, on the silica surface, the dissociation of the silanol groups is strongly promoted in the presence of PL, also causing σ_o to become more negative (fig. 4.4). The $-NH_3^+$ charges of PL are not titrated below pH 7, because proton titrations of PL adsorbed on PS latex between pH 3 and 7 did not show any change in α in this region. Hence σ_o is very likely to be solely due to the dissociation of silanol groups, also in the presence of adsorbed PL. It is remarkable that the σ_o(pH) curve of silica with adsorbed PL resembles that of borosilicate glass without PL. Both charge-pH curves are linear over a large pH range in contradistinction to the curve of bare silica (fig. 4.4). The charge development on the glass-water interface, which has a thick gel layer, is apparantly similar to that of the silica-water interface which has a much less porous double layer but which is covered with a polyelectrolyte layer (see chapter 5).

From the above it will be clear that only in the case of PS the ad-

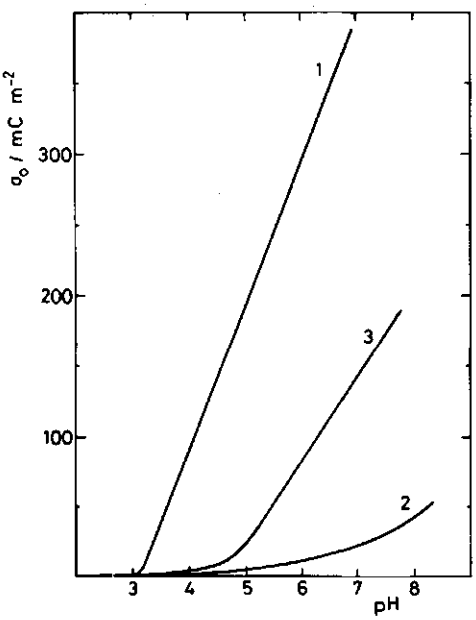


Fig. 4.4 Charge-pH curves of borosilicate glass B_2 and AEROSIL OX50 in 0.01 M NaBr. T = 293.15 K.
1. Borosilicate glass; 2. AEROSIL; 3. AEROSIL in the presence of excess PL-L (DP 192).

sorbed amount can be measured simple as a function of the surface charge. In the case of AgI and silica only the plateau value adsorption as a function of the pAg and pH respectively is measurable. The surface charge-pH (or pAg) curves in the presence of a surplus PL must also be known, for plotting the maximally adsorbed amount of PL as a function of the surface charge.

4.3.5.1 Polystyrene surface charge density and the effect of latex pretreatment

In fig. 4.5 adsorption isotherms of PL-L (DP 1682) on latex L (σ_o = -11 mC m^{-2}) and M_2 (σ_o = -60 mC m^{-2}) in 0.01 M NaBr are shown. There is a significant increase in the PL adsorption with increasing surface charge. Of course this is expected because of the attractive electro-

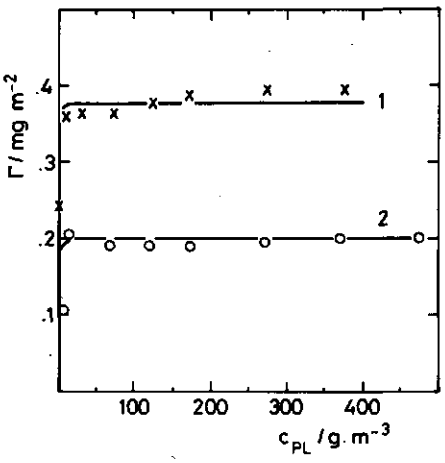


Fig. 4.5 Adsorption of PL-L (DP 1683) on PS (latices M_2 and L) in 0.01 M NaBr at pH = 6. T = 293 K.
x-x PS latex M_2 (σ_o = -60 mC.m^{-2}); o-o PS latex L (σ_o = -12 mC.m^{-2}).

static interaction between the $-OSO_3^-$ groups on the PS and the $-NH_3^+$ groups of the lysine side chains of PL.
In connection to the above it would be interesting to know whether or not the surface charge of a latex is altered due to a treatment with ion exchangers.

Most experiments with latex described in this thesis are performed with (mixed bed) ion exchanged (i.e.) latex. On the other hand *Norde (1976, 1978)* used latex that was only dialysed. The surface charge of Nordes latices was calculated from the loss in sulphur due to the i.e. treatment and the titration charge of the i.e. latex. For latex M (same formulation as used here) Norde found for an exchanged and unexchanged polystyrene latex, σ_o values of -45 and -84 mCm^{-2} respectively. Norde found also that on i.e. latices, the adsorption plataus of Human Plasma Albumine (HPA) are lower by about 25%. The difference in plateau adsorption (pH 4.0) between Nordes unexchanged high charge density latex (σ_o = -155 mC.m^{-2}) and Nordes low charge density latex (σ_o = -23 mC m^{-2}) was of the same magnitude: 30%.

In contrast with Nordes results we found that the plateau values of PL adsorption on polystyrene, before and after ion exchange, were the

same, despite of the clear effect of the surface charge on the adsorption of PL, as was shown in fig. 4.5. This indicates that the difference in surface charge due to i.e. treatment is small. This fact together with the observation that repeated i.e. treatments give the same value of σ_o (see 3.2.2), suggest strongly, that Nordes interpretation of the i.e. effect is not correct. An alternative explanation is that due to the i.e. treatment a layer of oligo and polystyrene sulphate molecules are stripped off from a PS particle as concluded by Norde, but that also in this way a new surface with new $-OSO_3^-$ groups, burried in the PS particle as ion pairs before the i.e. and hence not detectable by titration, is formed. The consequence would be that the values of σ_o used by Norde for the study of HPA and RNAse adsorption on PS particles are about 50% too high.

Although there is no effect of i.e. on the surface charge of PS, there exist an effect of the ionic strength history after polymerization of a latex on the σ_o value found. *Van den Hoven (1984)* found that the negative surface charge of PS particles increased by a factor 1.5-2 when a latex has been once exposed to an ionic strength of 0.1 M. He concluded this from conductometric titration of latex samples, that were brought in the deionized H^+ form, before and after exposure to 0.1 M KNO_3. A second exposure to 0.1 M electrolyte had no further effect on σ_o. The mechanism for this irreversible effect is not clear yet. Also from electrokinetic measurements on PS plugs *van der Put (1980)* and *van den Hoven (1984)* obtained indications for this electrolyte effect on σ_o. A more detailed discussion is given by *van den Hoven (1984)*. Because of the increase in the plateau adsorption value of PL with increasing surface charge on the PS, the adsorption plateau on electrolyte pre-treated latices will be higher than that on untreated PS surfaces.

Unfortunately no systematic measurements of PL adsorption on PS are available to quantify this. Most of the latices used in this study (except M_1) were not treated with 0.1 M electrolyte before use in adsorption studies. The consequences of this for the electrolyte dependence of the PL adsorption on PS, will be discussed in section 4.3.6.1. In conclusion it can be said that a PS surface with a better defined σ_o can be obtained when before i.e. first a salt treatment is given.

4.3.5.2 Silica and Borosilicate glass

Both silica- and pyrex glass are hydrophilic with a weakly acid character, because of the presence of silanol groups in the interfacial region. In the case of pyrex dissociation of the more strongly acid boranol groups can also contribute to the surface charge. Because of the presence of the weak acid groups the surface charge is pH- and ionic strength dependent. (e.g. *James and Parks, 1982*).

The parameter most relevant for the amount of PL adsorbed, is the electric potential at the place of the adsorbing PL segments. This potential depends on many factors such as the pH, ionic strength and the adsorbed amount itself. Its value is not known. In an experimental situation the adsorbed amount can be plotted as a function of the pH or the related surface charge.

In the case of oxides, which behave non-Nernstian, the relation between the solution pH and the surface potential must be a more complicated one than Nernst. Hence the surface potential cannot be easily calculated from the equilibrium pH in these cases.

Furthermore it is not the surface potential which is of relevance for the adsorption of PL segments on the surface, but the potential at some distance from the surface. This is because the charge at a polylysine segment cannot reach the surface due to the finite volume of the ionized groups. In the polyelectrolyte adsorption theory of van der Schee (see Chapter 7) the effective adsorption energy per segment $\chi_{s,eff}$ is defined as: $\chi_{s,eff} = \chi_s - (1-\alpha)e\psi_1/kT$ in which χ_s is the nonionic adsorption energy, α is the dissociation grade of the $-NH_3^+$ groups and ψ_1 is the potential at the place of the train segments.

Except chemical contributions (i.e. H-bonds and hydrophobic bonding) the non-ionic adsorption energy χ_s can contain contributions of dipolar interactions. In the case of charged polypeptides not only the displacement of water dipoles by charged groups can play a role but also the strong dipole of the peptide groups (μ = 3.4 D (*Wada, 1961*)) can compete with water dipoles at the surface. The magnitude of these contributions is determined by the surface charge, because the adsorption energy of a dipole is $\vec{\mu}\cdot\vec{E}$ and the field strength $\vec{E}$ at the locus of adsorption is according to Gauss law proportional to the surface charge σ_o. Hence the adsorption of polylysine is in part determined by the potential at the place were a segment adsorbs and in part by the surface charge.

Experimentally the amount of PL adsorbed on silica or glass can only be measured as a function of the suspension pH. The additional informa-

tion of the corresponding charge-pH curves (i.e. titration curves of silica in the presence of PL) is needed to construct the surface charge dependence of the adsorbed amount (see section 4.3.5). In many polyelectrolyte adsorption and/or flocculation studies the authors do not perceive this in discussing the influence of the surface charge. The thick gellayer (i.e. porous double layer) that exists in the case of glass is a complicating factor. The measured titration charge (see fig. 4.4), which is actually a space charge density, is not the charge density which the PL molecules 'see'. The charge in the gellayer can only be compensated for by micro-ions and not by large macromolecules such as PL. In the case of silica, which has only a thin gellayer, silanol groups can probably interact directly with $-NH_3^+$ groups of the PL.

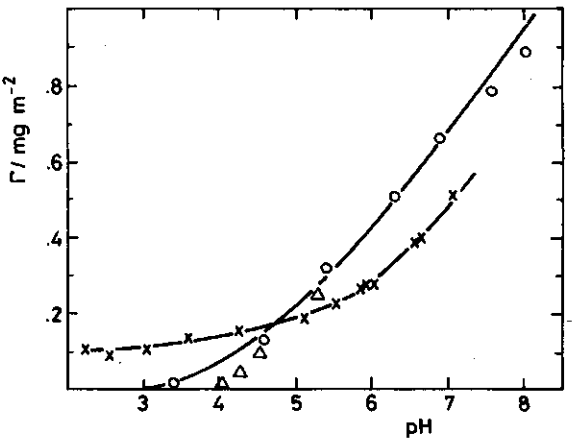


Fig. 4.6 Adsorption of poly-L-lysine as a function of the equilibrium pH. Electrolyte: 0.01 M NaBr; T = 293 K.
x-x PL-L (DP 1683)/Borosilicate glass B_2; o-o PL-L (DP 192)/AEROSIL OX50
Δ-Δ PL-L (DP 1683)/AEROSIL OX50.

In fig. 4.6 the plateau adsorption values of PL in 0.01 M NaBr (T = 293 K) on silica and glass powder are plotted against the solution equilibrium pH. The suspension effect on the pH measurements was probably small because the pH difference measured between the suspension and the equilibrium supernatant was always smaller than 0.02 pH units. From the figure one can see that at the p.z.c. of silica (pH ∿ 3) the adsorbed amount is also negligibly small. This is an indication that

electrostatic interactions between adsorbent and adsorbate are dominating in this case. The hydrogen bonding between R-C=O or R-NH of a peptide group and a surface silanol group is apparently not strong enough to give a measurable adsorption. However in the case of glass the adsorbed amount has a finite value at pH 3. The difference in plateau value at pH 3 between silica and glass is probably caused by the difference in p.z.c. between the two substrates, and not to a difference in hydrophobicity as one might think at first sight.
Jednačak et al. (1974) measured the i.e.p.'s of various glasses from streaming potential and/or streaming current measurements on glass plugs as a function of the pH and ionic strength. The value of the i.e.p. they found for (vitreous) silica is pH 3, whereas the value for pyrex glass is about pH 2.5. This difference has no consequences for the position of the proton titration curve of glass as explained before. Because of the flat charge-pH curves at low pH, a shift in the p.z.c. due to the interaction of the silica or glass with PL has almost no effect on the actual value of the surface charge density at pH 3. This is demonstrated in fig. 4.4 for the surface charge-pH curves of AEROSIL with and without added PL. Because of the large uncertainties in the specific surface area of the glass powder nothing more quantitative can be said about the difference in slope found between the Γ-pH curves of glass and silica. In the case of silica the slope of the Γ-pH curve

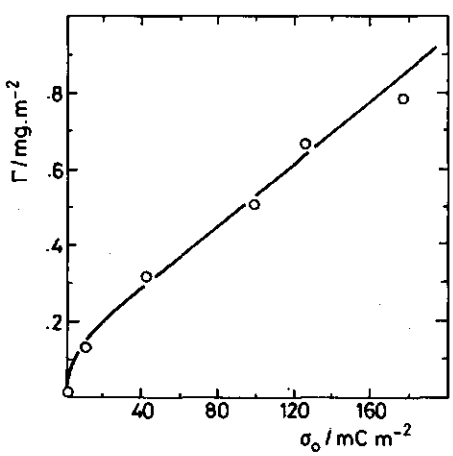


Fig. 4.7 Adsorption of poly-L-lysine (DP 190) on AEROSIL OX50 as a function of σ_o in 0.01 M NaBr. T = 293 K.

(110 mC/pH unit) is almost twice that of the proton titration curve (61 mC/pH unit). It is therefore very likely that the adsorption of PL on silica is superequivalent over the whole pH range studied.

In fig. 4.7 the adsorbed amount PL (DP 192) in 0.01 M NaBr is plotted against σ_o instead of the pH. At moderate σ_o values the amount of PL adsorbed increases almost linearly with the surface charge. This was also found by van der Schee for the adsorption of PL on AgI. Here however there is a definite value of the adsorbed amount PL at zero surface charge, probably because of the hydrophobic surface present in this case. The 'linear' behaviour is predicted by the polyelectrolyte adsorption theory of *van der Schee (1984)*.

4.3.6 Influence of electrolyte concentration

With polyelectrolyte adsorption the electric repulsion between similarly charged groups will oppose the formation of thick adsorbate layers. On the other hand, if this repulsion is suppressed by electrolytes Γ can become high.
When the non-ionic adsorption energy parameter χ_s is zero or small, there is a sensitive balance between the electrostatic attraction between surface and PL charges and the lateral electrostatic repulsion between adsorbed polyelectrolyte charges in loops and tails. It depends on this balance whether the adsorption increases or decreases upon increasing ionic strength.

4.3.6.1 Polystyrene latex and AgI

In fig. 4.8 the plateau adsorption values of PL.HBr on polystyrene and AgI respectively are plotted as a function of -log I. As expected the adsorption increases in both cases with increasing ionic strength I. At higher I the adsorption becomes also more sensitive to the molecular mass as already discussed in (4.3.4). For the quantitative interpretation of the effect of the ionic strength on the adsorbed amount it is necessary to realize that in the case of PS the surface charge above 0.1 M NaBr can be higher than at 10^{-3} M NaBr as discussed before (section 4.3.5.1). This will lead to a somewhat higher adsorption than would otherwise be at higher salt concentration. The increase in adsorbed amount in 0.1 M NaBr when σ_o is increased from -40 to -80 mC m^{-2} is only a few percent as predicted by the polyelectrolyte adsorption theory of van de Schee, under similar conditions as the experimental

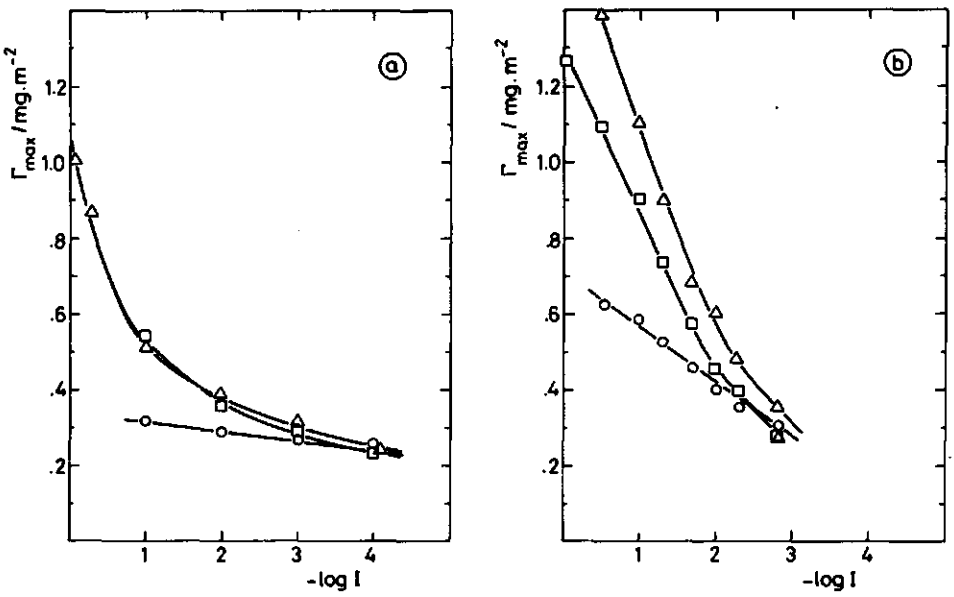


Fig. 4.8 Adsorption of PL (expressed in mg PL.HBr) as a function of the electrolyte concentration (i.e. -log(ionic strength NaBr)) on PS and AgI. T = 293 K.
1. PS (latex M_4): o-o PL-L DP 19: □-□ PL-DL DP 240; Δ-Δ PL-L DP 1683 electrolyte: NaBr; pH 6.
2. AgI: o-o ε-Acp-lys_{16}-NHMe; □-□ PL-L DP 300; Δ-Δ PL-L DP 2000 electrolyte: HNO_3; surface charge AgI $\sigma_o \tilde{\approx} 10$ mC.m^{-2}.

ones. The effect of the lateral interaction between adsorbed PL segments is probably dominant.

Considering the ionic strength dependence of the PL adsorption on AgI (*van der Schee, 1984; Bonekamp et al., 1983*) also in this case the experiments are not performed at constant surface charge, but at about constant pAg, so σ_o increases with increasing I. The magnitude of this effect is not exactly known because the charge-potential curves of AgI with adsorbed PL at different electrolyte concentrations are not available (only the curve of 0.1 M KNO_3). It will be of the same order of magnitude as with bare AgI, and probably less, when the situation may be compared with the silica-PL interaction. In the presence of PL the pAg is also not exactly constant when the ionic strength is varied, unless special precautions are taken, which was not the case here.

So the surface potential is not entirely constant either when the ionic strength is increased.

When at constant surface coverage of PL the ionic strength is increased, the surface charge also increases. However in the situation of fig. 4.8.2 the adsorbed amount of PL increases also as a direct consequence of increasing the ionic strength. This leads to a decrease in the pAg and consequently to a decrease in the surface charge. In other words, there are a number of trends that partly compensate each other. The approximation of constant surface charge is therefore probably reasonable in the situation of fig. 4.8.2. The comparison of the electrolyte dependence in the PL/PS (fig. 4.8.1) and PL/AgI (fig. 4.8.2) system, both on a basis of constant surface charge, seems therefore an acceptable first approximation, especially so since the lateral electrostatic interaction between the polymer segments is probably the dominant factor.

The adsorption of PL continuous to rise progressively beyond 0.1 M electrolyte both for AgI and latex as the substrate. The diffuse part of the electrical double layer is then however almost completely compressed ($\kappa^{-1}<1$ nm). For polyelectrolyte systems it is quite common however that the influence of the salt persists above 0.1 M. A qualitative explanation is as follows: The distance between the charges on a polyelectrolyte chain is usually very small, about 0.4 nm for PL and even smaller for fully charged polyacrylic acid (0.25 nm). Hence at 0.1 M salt were $\kappa^{-1} \approx 1$ nm two adjacent charges on a polyelectrolyte chain are still not completely screened. An example of this effect in a polyelectrolyte solution is the titration behaviour of weak acid or weak basic polyelectrolytes. A plot of pK_{app} versus degree of dissociation α is still not horizontal up to concentrations of 1 M electrolyte. The same is found by us for plots of the specific viscosity of PL solutions against PL concentration with NaBr concentrations above 0.1 M NaBr. In the domain of polyelectrolyte adsorption, *Greene (1971)* found a strong increase in the plateau value above 0.1 M NaCl for the adsorption of poly(2-sulphoethylmethacrylate) on polyethylene powder. *Marra et al. (1982)* reported adsorptions of polystyrene sulphonate on silica which kept increasing at least up to 3 M $MgCl_2$ or NaCl. The polyelectrolyte adsorption theory of *van der Schee (1984)* is capable of explaining this rise of adsorption with increasing I (see also chapter 7). It seems therefore not necessarily to assume an increase of χ with rising salt concentrations to explain the electrolyte dependence on the adsorption at high electrolyte concentrations. It is there-

fore concluded that the strong increase of the adsorbed amount above 0.1 M electrolyte is still due to the screening of the $-NH_3^+$ charges.

It is interesting to note that polylysine-DNA complexes dissociate in 1.0 M electrolyte solution despite the presence of H-bonds between the lysine residues and phosphate groups of the DNA (*Helene and Maurizot, 1981*). A similar situation exists with the PL-silica system as will be shown later (section 4.3.6.2). Obviously in these cases the binding is predominantly of an electrostatic character and hence it is reduced by the addition of indifferent electrolytes.
On the other hand with PL adsorbing on PS and AgI, non-coulombic attractive interactions, such as hydrophobic bonding, contribute significantly to the adsorption energy per segment, as already was suggested when the adsorption isotherms were discussed (section 4.3.4).

As one can see from fig. 4.3.1, no significant differences between the behaviour of PL-L and PL-DL, with respect to the electrolyte influence could be detected as expected (see also section 4.3.4), at least for electrolyte concentrations below 0.1 M.

The adsorption of PL on polystyrene is reversible with respect to changes in ionic strength. This reversibility was tested as follows: After adsorption of PL-DL (DP 240) on polystyrene latex M in 0.01 M NaBr, the supernatant obtained after mild centrifugation was replaced by a known volume of NaBr solution in such a way that the final NaBr concentration was $\sim 10^{-4}$ M. The latex was then resuspended and rotated end over end for 16 hours. The desorbed amount was determined and appeared to be equal to the difference in plateau value adsorption between 10^{-2} and 10^{-4} M NaBr.

SCN^- ions are known to bind specifically onto the $R\text{-}NH_3^+$ side chain groups of polylysine (*Conio et al., 1974*). This causes a reduction of the effective charge density on a polylysine chain (*Conio et al., 1974*) and an effect on the amount of PL adsorbed on PS in the presence of NaSCN is therefore expected. This was indeed found: The plateau adsorption value of PL-L (DP 1683) on latex M_2 in 0.01 and 0.1 M NaSCN is respectively 0.48 and 0.71 $mg.m^{-2}$. The corresponding values in 0.01 and 0.1 M NaBr (fig. 4.3) are respectively 0.36 and 0.46 mg m^{-2}. The plateau adsorption values of PL adsorption on PS with NaCl or $NaNO_3$ as the electrolyte are not different from the isotherms found with NaBr, as expected. From the effect found with SCN^- it is concluded that NaSCN acts as a screener of the interaction between PL segments more effective than NaBr. This stronger screening more than compensates the expected weakened $-NH_3^+$ surface $-OSO_3^-$ attraction. In the case of si-

lica as adsorbent this is not so, because here the amount of PL-L adsorbed is about the same whether NaBr or NaSCN is used (see fig. 4.3a).

4.3.6.2 Silica and Borosilicate glass

Silica and borosilicate glass are hydrophilic adsorbents. The non-ionic adsorption energy χ_s of PL does not contain a contribution of hydrophobic bonding in these cases. Furthermore the strong competition of PL segments with water molecules for surface sites will lower the value of χ_s. Therefore χ_s will be lower than in the case of AgI and PS. Consequently the balance between the electrostatic attraction between surface charges and PL charges and the lateral electrostatic repulsion between adsorbed polyelectrolyte charges in loops and tails, will be more sensitive for electrolyte than in the case of PS and AgI.

When known amounts of a NaBr and a PL solution in demi water (pH 6) are added in the given order to a weighted amount of purified borosilicate glass powder, no significant differences in the plateau values are found on varying the ionic strength of the suspension (see fig. 4.9). Clearly several opposing effects play a role in these system. First, the pH of the glass suspensions is not constant, but decreases with increasing ionic strength as one can see in fig. 4.9. This decrease

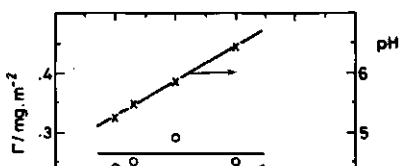


Fig. 4.9 Adsorption of PL-L (DP 1683) on Borosilicate glass B_2 as a function of the ionic strength. T = 293 K.
o-o Γ vs -logI for $5.2 < pH_{ads} < 6.5$ (pH_{ads} indicated as x)
□-□ Γ vs -logI for $3.2 < pH_{ads} < 4.3$ (pH_{ads} indicated as Δ)
Note the different scales.

is expected because the apparent pK of the silanol groups decreases with increasing ionic strength. The amount of PL adsorbed at constant ionic strength, decreases also with decreasing pH (see fig. 4.6). Apparently the effect of the increasing ionic strength compensates the effect of the decreasing pH. At higher ionic strength the competition between Na^+ ions and $-NH_3^+$ groups of the PL starts to suppress the adsorption of PL. At low pH values, the amount of PL adsorbed increases slightly with increasing ionic strength, despite the decrease in pH occurring when the electrolyte concentration was increased. As under these conditions the effect of pH on σ_o is small, this trend is probably entirely attributable to the reduction of the screening between the charged $-NH_3^+$ groups of PL.

In order to investigate the effect of ionic strength on the adsorption plateau values of PL on substrates with weakly acidic surface groups, it is better to measure the pH dependence of the adsorption at several ionic strengths and to construct from these data the electrolyte dependence at constant pH. This was done by us for the adsorption of PL on silica. The use of buffers has to be avoided because of the possible interference with the silica-PL system.

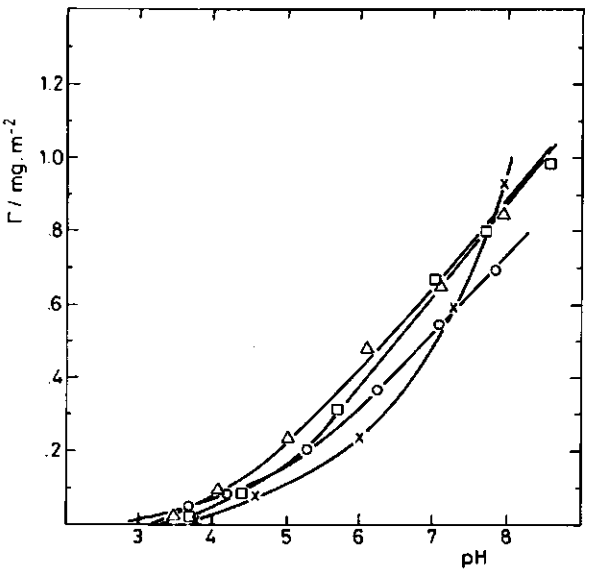


Fig. 4.10 Effect of the pH on the adsorption of PL-DL (DP 307) at different ionic strength on silica (AEROSIL OX50), T = 293 K.
o-o 10^{-3} M NaBr; Δ-Δ 10^{-2} NaBr; □-□ 10^{-1} M NaBr; x-x 0.3 M NaBr.

In fig. 4.10 the plateau values of the adsorption of PL-DL (DP 307) on silica are plotted as a function of the pH at several values of the ionic strength. The curves at diffrent ionic strength were measured simultaneously in one series of experiments, with the objective to use the same stock solutions and pipettes for each measurement, so that smaller differences in adsorbed amount between two curves could be estimated. Series of curves with PL-L (DP 240) and PL-L (DP 192) were obtained in the same way. They showed similar behaviour as PL-DL (DP = 308) in fig. 4.10. This indicates that the ionic strength dependence of the Γ vs pH curves is reproducible within one set of curves, and that it is independent of the molecular mass and stereoregular configuration of the PL.

At each ionic strength value, the amount of PL adsorbed increases with increasing pH due to the increasing surface charge as already discussed under (4.3.5.2). In fig. 4.11 the plateau values of the adsorption of PL-DL as a function of -log I are plotted for three pH values (i.e. vertical cross sections through the set of curves in fig. 4.10). At pH values lower than 4, the adsorbed amount seems to decrease monotonically with increasing ionic strength. The experimental error is however too large at these low adsorbed amounts to allow conclusions to be drawn

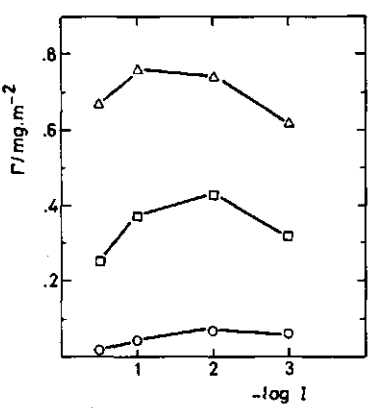


Fig. 4.11 Influence of the ionic strength I on the adsorption of PL-DL (DP 307) on AEROSIL OX50 at different pH values.
o-o pH 4.0; □-□ pH 6.0; Δ-Δ pH 7.5.

from the observed trend. The lower adsorbed amount at 0.3 M NaBr compared to that at 10^{-3} M NaBr is probably real. At higher pH values the Γ vs -log I curves show a maximum, indicating that the factor dominating the adsorbed amount is different at high and low ionic strength.

With the help of the σ_o-pH curves of silica in the presence of excess PL for 10^{-3}, 10^{-2} and 10^{-1} M NaBr (see fig. 4.12), the adsorbed

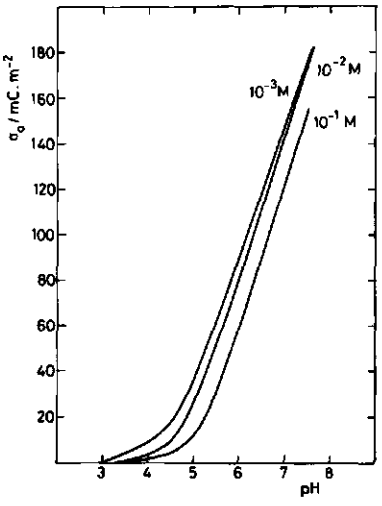


Fig. 4.12 Charge-pH curves of AEROSIL OX50 silica in the presence of excess PL (Γ_{max} at each pH). T = 293.15 K. c_{sil} = 2.50% (w/v). Electrolyte concentrations are indicated.

amount as a function of pH can be replotted as a function of σ_o for the ionic strength values indicated. From this set of curves the amount of PL adsorbed as a function of -log I at constant σ_o values as shown in fig. 4.13 are observed. It can be seen from these curves that at constant surface charge the plateau adsorption of PL only increases with increasing ionic strength and no maximum is present anymore. In contrast with the comparable curves for AgI and PS as the substrates for PL, the Γ vs -log I (σ_o = constant) curves for silica are concave and the increase above 0.01 M salt is only small. This shows that the balance between the two opposing salt effects mentioned in the beginning of this section, is turned in favour of the weakening of the $-NH_3^+/\equiv Si-O^-$ attraction at higher salt concentration. With the PL - glass system at low pH a similar effect was found. The displacement of $-NH_3^+$ from surface sites by Na^+, at higher ionic strength, can play a role in the

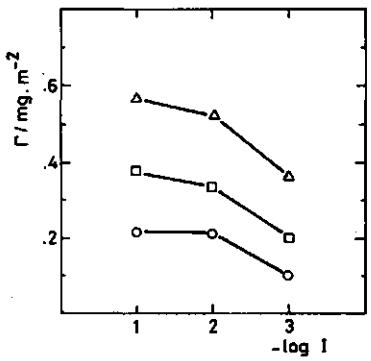


Fig. 4.13 Influence of the ionic strength on the adsorption of PL-DL (DP 307) on silica at constant surface charge.
o-o σ_o = -20 mC m^{-2}; □-□ σ_o = -50 mC.m^{-2}; Δ-Δ σ_o -100 mC.m^{-2}.

observed trend. It is stressed again that such a salt effect, as observed in fig. 4.13, can only be found when the non-ionic adsorption energy χ_s is a minor factor contributing to the binding.

The problem remains how to explain the maximum in the Γ vs -logI curves at constant pH (fig. 4.11). The reason must be sought in the fact that, in contradistinction to the behaviour of bare silica, the surface charge at constant pH of silica with excess PL present, is a decreasing function of the ionic strength. This is another indication for the competition between Na^+ and $-NH_3^+$ charges of PL, for dissociated surface silanol groups. A more detailed discussion of the charge-pH curves of silica will be given in chapter 5. It is probably this drop in surface charge, which causes a decrease in the adsorbed amount at higher ionic strength at constant pH. It would be interesting to calculate the electrolyte dependence of the adsorption of positively charged polyelectrolytes on negatively charged surfaces for χ_s values about or less than $\chi_{s,cr}$ (see chapter 7). In this respect it is interesting that *Lindquist (1975)* found an increase in the amount adsorbed at constant pH for polyethyleneimine on silica (Ludox AM) particles up to 0.1 M NaCl. This is probably due to a higher χ_s value in this case.

4.3.7 The stability of latex and AgI sols against low molecular mass electrolyte in the presence of polylysine

When a negatively charged solid dispersed in water is brought into contact with an increasing concentration of a cationic polymer, three zones of action may be recognized, viz.(i): a stable zone at very low total polymer concentration (i.e. very low polyelectrolyte coverage of the particles), (ii) a flocculation* zone and (iii) at higher polyelectrolyte contents (high surface coverage) a zone of restabilization. The unstable region is bound by the critical flocculation concentration (CFC) and the restabilization concentration (RSC). The concentration of polyelectrolyte required for maximum destabilization is defined as the optimum flocculation concentration (OFC).

The main reason for flocculation of charged particles by oppositely charged polyelectrolytes is charge neutralization (*Gregory, 1973; Ho and Howard, 1982; Lindquist, 1975; Bleier and Goddard, 1980*). Besides the extent of adsorption, also the configuration of the adsorbed polymer determines whether flocculation or stabilization occurs. The coagulation concentration is also dependent on the structure of the adsorbed layer. *Corry and Seaman (1978)* pointed out that in the PL/latex system, polylysine can act either as a flocculating or stabilizing agent, depending on the configuration and disposition of the adsorbed polymer.

In this study, the stability of polystyrene- and AgI-particles covered with PL above the RSC against low molecular mass electrolyte was investigated. The influence of the PL molecular mass was also considered. In this way some additional information can be obtained about the configuration of the adsorbed polylysine. The stability measurements were done by a static method described in section 4.2.5.

The stability of polystyrene and AgI sols, containing particles covered with PL-L, against increasing KNO_3 concentration was measured. In the absence of PL the coagulation concentration of PS latex was not measurably different with KNO_3 or NaBr as electrolyte. Also in the presence of PL, the same trends were found with KNO_3 or KBr as electrolyte.

---

* The term flocculation is used here when destabilization occurs by the addition of polyelectrolyte. The term coagulation is used in the case of destabilization by low molecular mass electrolyte.

Experiments with sols in the presence of adsorbed PL were performed by us under conditions of large excess (i.e. with respect to the adsorbed amount) PL in the bulk (c_{PL} > 30 g.m^{-3}) or small initial excess PL in the bulk (c_{PL} < 0.7 g.m^{-3}). As the adsorbed amount increases with increasing ionic strength, the concentration PL in the bulk solution decreases with increasing ionic strength to very low values in the latter case. Above 0.1 M electrolyte, plateau adsorption is not completely reached in the case of small initial excess PL. When large excess is present, the PL concentration in the bulk stays about constant on increasing the electrolyte concentration. In this situation the plateau values are largely reached at each salt concentration.

In fig. 4.14 and 4.15 the relative turbidity τ_r, i.e. the measured extinction of the supernatant devided by the turbidity of the corresponding stable blanc sol τ_o, is plotted as a function of the KNO_3 concentration for AgI- and PS sols, both bare and covered with PL-L of different molecular masses. In both cases the total concentration PL ensures large excess of PL-L. From fig. 4.15 one can see that the stability of the AgI sol increases with increasing molecular mass of the adsorbed PL-L. In the case of PL-L (DP 19) there is apparently no extra gain in stability of the sol, despite the increasing adsorbed

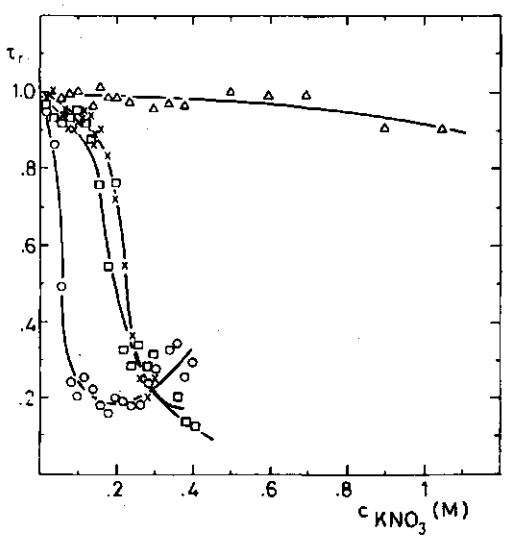


Fig. 4.14 Stability curves of PS (latex M_2) with and without added PL-L at pH 6 and T = 298.15 K. c_{PL}^T = 30 g.m^{-3}. x-x PS latex M_2; o-o PS latex M_2/PL-L (DP 19); □-□ PS latex M_2/PL-L (DP 240); Δ-Δ PS latex M_2/PL-L (DP 1683).

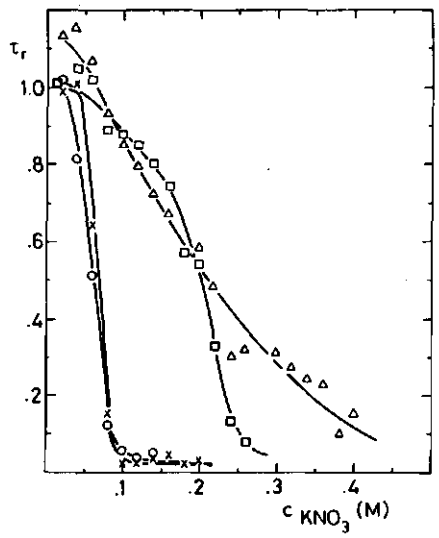


Fig. 4.15 Stability against 1-1 electrolyte of AgI sol with and without PL-L.
T = 298.15 K, c_{PL}^{T} = 50 $g.m^{-3}$, pH 6.
x-x Bare AgI; o-o AgI/PL-L (DP 19); □-□ AgI/PL-L (DP 192); Δ-Δ AgI/PL-L (DP 1683).

amount when the salt concentration is raised. However, in the presence of large excess PL-L (DP 192) or PL-L (DP 1683), the AgI sols are more stable than bare AgI sols. Apparently loops and tails are formed at higher ionic strength, large enough to give some steric stabilization in these cases.

In the case of small initial excess PL (see fig. 4.16) no evidence for steric stability was found. Under these conditions the sols are purely electrostatically stabilized. The effect of the PL concentration in the bulk on the sol stability is shown in fig. 4.16.
There is an increasing stability when at each KNO_3 concentration the volume fraction PL in the bulk is such, that the plateau adsorption is largely reached. In those cases where the volume fraction in the bulk ϕ_*, is very low, thus resulting in amounts of adsorption just below the plateau or even less, on increasing the KNO_3 concentration (c_{PL}^{T} = 1.0 $g.m^{-3}$), there is also no appreciable molecular mass dependence of the stability (see fig. 4.17). Hence the conformation of the adsorbed PL layer is more flat under these conditions than in the case

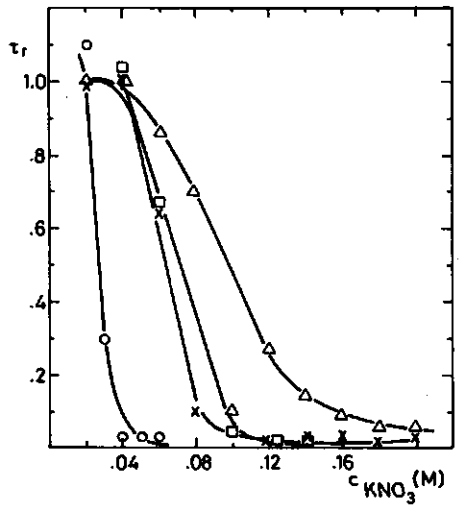


Fig. 4.16 Influence of the total PL (DP 62) concentration on the electrolyte stability of AgI sol, pI = 4, T = 298.15 K.
x-x bare AgI; o-o AgI/PL-L C_{PL}^{T} = 1 g.m.$^{-3}$; □-□ AgI/PL-L C_{PL}^{T} = 5 g.m^{-3}; Δ-Δ AgI/PL-L C_{PL}^{T} = 50 g.m^{-3}.

of large excess PL. So in the case of small initial excess PL the possibility of significant steric stability no longer exists. The results obtained by us under conditions of small initial excess polylysine are consistent with those of *van der Schee (1984)*, who measured the electrolyte stability of PL-covered AgI sols, under similar conditions by a kinetic method.

The stability curves of PL-covered PS particles at large excess PL as a function of the PL molecular mass, are qualitatively the same as those with AgI (fig. 4.14), however the effect of the PL molecular mass is here much more pronounced. The PS sols with particles covered with PL-L (DP 1683) are completely stable up to electrolyte concentrations of at least 1.0 M. A complicating factor here is the PS surface itself. The PS-water interface is to some extent 'hairy' (see section 3.2.3). This causes a kind of intrinsic steric stability of the latex. Coagulation concentrations of latex in the presence of PL (DP 19) and PL (DP 240) are even lower than those of bare latex. This indicates that the interfacial PL layer is more flat than the bare PS-water inter-

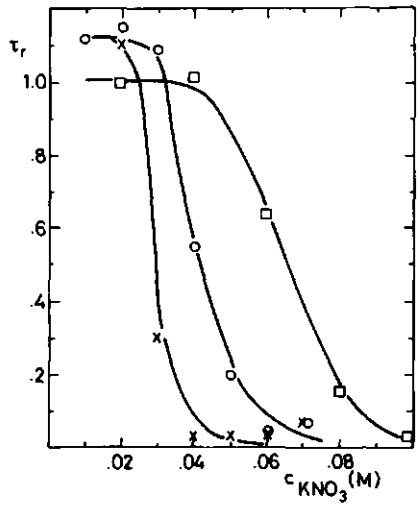


Fig. 4.17 Stability of AgI sol as a function of the KNO_3 concentration. No excess PL-L present. c_{PL}^T = 1.0 $g.m^{-3}$, T = 298.15 K, pI = 4.
a-x AgI/PL-L (DP 62); o-o AgI/PL-L (DP 192); □-□ bare AgI.

face. On the other hand with PS covered by PL-L (DP 1683) this is clearly not the case. Quantitative differences in the stability behaviour of AgI- and PS sols covered with PL are possibly caused by differences in Hamaker constant between these substances. Also other factors such as differences in sedimentation velocity of the aggregate and adsorbed amount, between the two substrates can contribute to it. The stability of PS latex in the presence of PL is consistent with electrokinetic measurements performed by us on PL-coated PS plugs. The results obtained here are qualitatively in agreement with those obtained by *Williams et al., (1982)* for negatively charged $BaSO_4$ particles, covered with polyacrylic acid. *Corry (1978)* measured the rate constant of the flocculation of PS sols at a constant low molecular mass salt level of 0.1 M NaCl as a function of PL coverage (the electrophoretic mobility of PS particles was taken as a measure of surface coverage). The rate constant at high surface coverage was appreciably lower than that at moderate surface coverage. *Corry* concluded that at high surface coverage the low aggregation rate constant must be accounted for by steric stabilization, which is in line with our experience. No satisfying

explanation was offered for the extra stability gain at very low surface coverage. In my view the unexpectedly high rate constant found by *Corry* must be due to the haryness of the PS particles themselves, a feature similar to that found by us.

4.4 SUMMARY AND CONCLUSIONS

In this chapter adsorption properties of highly charged polylysine (pH < 6) on hydrophobic (polystyrene and AgI) and hydrophilic (borosilicate glass and silica) adsorbents were discussed.

In all cases high affinity isotherms are found in which the plateau adsorption is less than or about monolayer coverage. At low ionic strength there is no effect of the molecular mass of PL on the adsorption, indicating flat adsorbate layers. No differences between the adsorption of PL-L and PL-DL are present. In most cases the adsorption of PL is superequivalent.

At low ionic strength (i.e. $I < 10^{-2}$M) the formation of thick adsorbate layers is prevented by the strong electrostatic repulsion between adsorbed PL segments, as is usually found for highly charged polyelectrolytes. As this electrostatic repulsion dominates the adsorption at low ionic strength, differences in adsorbed amount between the substrates used (at the same surface charge) are not very pronounced. Also the composition of the side chain of the cationic polyaminoacids has no influence under these conditions.

As expected the adsorption of polylysine increases with increasing negative surface charge. The adsorbed amount increases also when the ionic strength is increased, because then the repulsion between polymer segments is screened. The salt dependence was found to be reversible. On increasing the ionic strength, differences in adsorption behaviour between hydrophobic and hydrophilic adsorbents as the substrate for PL become visible. With silica the increase of the adsorbed amount PL levels off above 0.01 M NaBr, while on PS and AgI the adsorption progressively increases at least up to 1.0 M electrolyte. This suggests that χ_s is less in the case of silica. The reason for this can be the absence of hydrophobic interactions between PL segments and the surface, while in the case of AgI and PS hydrophobic interactions between the $-(CH_2)_4-NH_3^+$ side chain and the surface are possible.
In other words, the balance between the segment-surface attraction and the electrostatic segment-segment repulsion is turned in favour of weakening of the segment-surface interaction at high ionic strength

in the case of silica. This shows that the electrostatic contribution to $\chi_{s,eff}$ is relatively more important in the case of adsorption of PL on silica than in the case of PL-adsorption on AgI and PS.

The electrolyte stability of AgI and PS sols in the presence of adsorbed PL was also investigated. When the adsorbed amount at the coagulation concentration is just below the plateau value the sols are only charge stabilized, independent of the degree of polymerization of the PL. In the case of large excess PL, where the plateau value at the coagulation concentration is largely reached, the sols with PL (DP 192) and PL (DP 1683) are more stable than sols with bare particles. This together with the fact that at high ionic strength ($I > 0.1$ M) the adsorbed amount increases with increasing DP shows, that under these conditions the adsorbate layer is less flat and that more pronounced tails and loops must be present than at low ionic strength or adsorption values just below the plateau value.

4.5 REFERENCES

Abendroth, R.P. (1970). J. Colloid Interface Sci. *34*, 591-596.

Applequist, J. and Doty, P. (1962), in 'Polyaminoacids, Polypeptides, and Proteins', Stahmann, M.A. ed., Univ. Wisconsin Press, Madison, p. 161-177.

Ardizzone, S., Formaro, L. and Lyklema, J. (1982). J. Electroanal. Chem. *133*, 147-156.

Bleier, M. and Goddard, E.D. (1980). Colloids and Surfaces, *1*, 407-423.

Bonekamp, B.C., Schee, H.A. van der and Lyklema, J. (1983). Croat. Chem. Acta *56*, 695-704.

Brant, D.A. and Flory, P.J. (1965). J. Amer. Chem. Soc. *87*, 2788-2791.

Cafe, M.C. and Robb, I.D. (1982). J. Colloid Interface Sci., *86*, 411-421.

Conio, G., Patrone, E., Rialdi, G. and Ciferri, A. (1974). Macromolecules *7*, 654-659.

Cohen Stuart, M.A. (1980). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Cohen Stuart, M.A., Scheutjens, J.M.H.M. and Fleer, G.J. (1980). J. Polym. Sci., Polym. Phys. Ed. *18*, 559.

Corry, W.D. and Seaman, G.V.F. (1978). J. Colloid Interface Sci. *63*, 136-150.

Corry, W.D. (1978). J. Colloid Interface Sci. *63*, 151-160.

Eggert, A.R. (1976). Ph.D. thesis Lawrence University, Appleton Wisconsin.

Furusawa, K., Norde, W. and Lyklema, J. (1972). Kolloid Z.Z. Polym. *250*, 908-909.

Flory, P.J. (1969). 'Statistical mechanics of chain molecules' Interscience Publishers, Wiley and Sons, New York.

Greene, B.W. (1971). J. Colloid Interface Sci. *37*, 144-153.

Gregory, J. (1973). J. Colloid Interface Sci. *42*, 448-456.

Hesselink, F.Th. (1983) in 'Adsorption from solution at the solid-liquid Interface', Parfitt, G.D., Rochester, C.H., eds., Acad. Press London.

Helene, C. and Maurizot, J.C. (1981). CRC Crit. Rev. Bioch., 213-258.

Ho, C.H. and Howard, G.J. (1982) in 'The effect of polymers on dispersion properties', Tadros, Th.F. ed., Acad. Press, London-New York.

Horn, D. (1978), Progr. Colloid Polym. Sci. *65*, 251-264.

Horn, D. and Melzer, J. (1978). Fibre-Water Interaction Pap. Making Trans. Symp. ser. *1*, p. 135-150.

Hoven, Th.J.J. van den (1984). Doctoral thesis Agricultural University Wageningen, The Netherlands. In press.

James, R.D. and Parks, G.A. (1982) in 'Surface and Colloid Science' *12*, 119-216. Matijević, E. ed., Plenum Press New York-London.

Jednačak, J., Pravdić, V. and Haller, W. (1974). J. Colloid Interface Sci. *49*, 16-23.

Koopal, L.K. (1978). Doctoral thesis Agricultural University Wageningen, Commun. Agric. Univ. Wageningen, 78-12.

Kasper, R.D. (1971). Ph.D. thesis California Institute of Technology, Pasadena, California.

Lindquist, M.G. (1975). Ph.D. thesis Lawrence University Appleton Wisconsin.

Lindquist, M.G. and Stratton, R.A. (1976). J. Colloid Interface Sci. *55*, 45-59.

Marra, J., Schee, H.A. van der, Fleer, G.J. and Lyklema, J. (1983) in 'Adsorption from solution'. Ottewill, R.H., Rochester, C.H. and Smith, A.L., Eds., Acad. Press, 245-258.

Nyilas, E., Chiu, T-H. and Lederman, D.M. (1976) in 'Recent advances in Colloid and Interface Science'. Kerker, M., Acad. Press New York.

Norde, W. (1976). Doctoral thesis Wageningen, Commun. Agric. Univ. Wageningen, 76-6.

Norde, W. and Lyklema, J. (1978). J. Colloid Interface Sci. *66*, 257-265.

Put, A.G. van der (1980). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Rawls, H.R., Bartels, T. and Arends, J. (1982). J. Colloid Interface Sci., *87*, 339-345.

Schee, H.A. van der (1984). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Schee, H.A. van der, and Lyklema, J. (1982) in 'The effect of polymers on dispersion properties', Tadros, Th.F. ed., Acad. Press London-New York.

Terayama, H. (1952). J. Polym. Sci. *8*, 243-253.

Wada, A. (1961) in 'Polyaminoacids, Polypeptides and Proteins', Stahmann, M.A., ed., Univ. Wisconsin Press, Madison. 131-146.

Williams, P.A., Harrop, R., Philips, G.O., Pass, G. and Robb, I.D. (1982), J. Chem. Soc., Faraday Trans. 1, *78*, 1733-1740.

5 INTERACTIONS BETWEEN NEGATIVELY CHARGED COLLOIDAL PARTICLES AND POLYCATIONS

5.1 INTRODUCTION

Interactions between oppositely charged (biological) macromolecules play definite roles in biological and technological systems, for instance in the contact between connective tissues (*Gelman et al., 1973*). Also in many biochemical, biomedical and technological applications, complexes between oppositely charged macromolecules are of great importance. A biochemical application is, adsorption chromatography of biopolymers. The interaction of charged salivary proteins with hard tooth tissue (*Juriaanse, 1980*) and the binding of histone proteins to DNA (*Record et al., 1978*) are other examples. Flocculation of active sludge with oppositely charged polyelectrolyte is of importance in water purification (*Daniels, 1980*). Also the use of polyelectrolyte-complex material for ultrafiltration and dialysis membranes for medical purposes can be mentioned here (*Philipp, 1982*).

Negatively charged silica and polystyrene particles can be regarded as rigid spherical polyelectrolytes. This is because of the existence of fixed discrete charged groups on the particle surfaces which are relatively close together (see for example *Rice and Nagasawa (1961)* or *Tanford (1965)*).

In this chapter I will consider the adsorption of the flexible polyelectrolyte poly-L-lysine.HBr on polystyrene (PS) or silica particles as a special case of the formation of a polyelectrolyte complex (i.e. polycation-polyanion complex), rather than as a special case of uncharged polymer adsorption, as is usually done in the polyelectrolyte adsorption field.

The polyelectrolyte complex approach of polyelectrolyte adsorption was also suggested by *Horn (1978)* and *Philipp (1982)*.
The results obtained here will be compared with the properties of the more 'regular' polycation-polyanion complexes like, for instance the polylysine-polyglutamic complex (*Domard and Rinaudo, 1981*). It must be kept in mind, however, that the charged macromolecule charged particle systems considered here are in fact heterogeneous systems, while the polycation-polyanion complexes, are homogeneous one phase systems, at least at low concentrations, i.e. below the solubility limit of the complex.

5.2 EXPERIMENTAL

5.2.1 Materials

The materials used in this study: polyvinylsulphate-potassiumsalt (ICN inc.); polylysine.HBr (Sigma), poly-L-histidine (M = 15.000; Sigma); PS Latex and silica (AEROSIL OX50 Degussa) have been described in sections 3.1-3.3 and 4.2.1. The concentration of aqueous PL.HBr solutions calculated on a weight basis was in good agreement with the values found from a conductometric titration with calibrated $AgNO_3$ or hydrogen polystyrene sulphate (HPSS) solutions.

The preparation of HPSS from NaPSS (Waters ass.) was done by dialysis against excess HCl solutions and demi water in the given order. The PSS-residual concentration was then determined by a conductometric proton titration.

Conductivity water and analytical grade chemicals were used in all experiments.

5.2.2 Potentiometric and conductometric titrations

All conductometric and potentiometric measurements were performed in a well-closed double walled vessel, thermostatted at 293.15 ± 0.05 K, under a CO_2-free water vapour saturated N_2 atmosphere. Proton titrations for the surface charge determination of PS (latex M) have been described in section 3.2.2.

Proton titrations of silica in the presence or absence of PL.HBr and the construction of charge-pH curves from the obtained data have been described in section 3.3.3 and section 4.2.4.

Conductometric titrations of 20.0 cm^3 PS latex (1% (w/v)) or AEROSIL OX50 (1.25% (w/v)) with poly-L-lysine.HBr (0.02 rM) were performed with a Retch conductivity meter operating at 4 KHz.

Conductivity cells with cell constants of 10.4 m^{-1} or 71.9 m^{-1} were used. Additions of PL.HBr were done with an Agla microsyringe. Conductometric titrations at high pH values were performed with latex and PL.HBr solutions that before the titrations were brought to the same pH under a N_2 atmosphere with 1.0 M NaOH. The Agla microsyringe was then filled with PL.HBr (high pH) without allowing contact of the PL.HBr solution to the air.

5.2.3 Adsorption measurements

Adsorption isotherms of PL.HBr on the substrates PS (latex) or silica were determined by depletion as has been described in section 4.2.3. The concentrations of positively charged polylysine.HBr after adsorption were determined by titration with the potassium salt of polyvinylsulphate using toluidine blue as the indicator (*Terayama, 1952; Horn, 1978*). See also section 4.2.2 and 4.2.3.

5.2.4 Stability measurements

The stability of silica sols as a function of the degree of PL.HBr coverage of the particles was determined as follows: In 10 cm^3 sovirel tubes with screw cap (Teflon seal) 4.0 cm^3 silica sol (1.25% (w/v)) was added. Then 4.0 cm^3 PL.HBr with the desired concentration was added by means of a pipetman dispensing pipet, so that rapid mixing due to the PL.HBr solution jet was achieved. In this way a series with increasing total PL.HBr concentration and constant desired micro electrolyte concentration was prepared. The tubes were rotated end over end for 5 hours (at 295.6 ± 0.3 K) to obtain adsorption equilibrium. Then the tubes were incubated at this temperature without agitation for another 18 hours. After this time the OD (550 nm) of the top two cm^3 of the supernatant was measured. The results were plotted as the OD_{550nm}^{10mm} against the total polylysine concentration. Also the amount PL adsorbed of each sample and the pH was measured after the OD measurements.

5.2.5 Microcalorimetry

Some preliminary measurements of the heat of adsorption in the PL.HBr-PS latex system were performed in a LKB twin microcalorimeter. The procedure followed was the same as that described by Norde (1976, 1978). The PL.HBr-L and latex was dialyzed against the same electrolyte solution before the measurements. Due to the twin principle of the calorimeter the heat of dilution of the PL.HBr solution was automatically accounted. The heat of dilution of the latex was negligibly small.

5.2.6 UV and CD spectroscopy

Some attempts were undertaken to measure the UV and CD spectra of poly-L-histidine, adsorbed on silica. Instead of PL, PHis was chosen

because this polyaminoacid shows a conformational transition in solution between pH 4 and 6. (*Beychok, 1967*). UV (difference) spectra were recorded on a Beckman model 3600 UV spectrophotometer. Circular Dichroism spectra were recorded at a Jouan Roussel dichrograph under N_2 flush.
Matched quartz cuvettes with an optical pathway of 10.0 mm were used in most of the experiments.

5.3 RESULTS AND DISCUSSION

5.3.1 Adsorbed amount PL.HBr from depletion measurements

One of the experimental advantages of the polylysine/charged particle systems is, that the complex (i.e. adsorbed PL.HBr) in equilibrium with PL.HBr in solution, can easily be separated by centrifugation. The composition of the complex as a function of the equilibrium concentration PL.HBr (i.e. the adsorption isotherm) can then be obtained by determining the equilibrium concentration PL.HBr in the clear supernatant and calculation of the depleted amount of PL.HBr.
For the heterogeneous systems studied here it is most convenient to express the composition of the complex in µmol lysine $-NH_3^+$ charges adsorbed per m^2 particle geometrical surface area, instead of the ratio $-NH_3^+$ and $-OSO_3^-$ or $Si-O^-$ charges in the complex as is usually done for polycation-polyanion complexes.

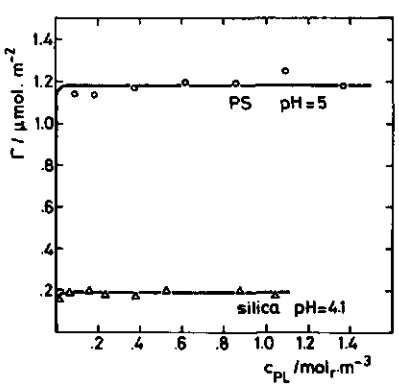


Fig. 5.1 Adsorption of PL.HBr (DP 192) on PS (latex M_3) and silica (AEROSIL OX50) from conductivity water (no added electrolyte) T = 293 K
o-o PL.HBr/PS σ_o = -0.54 µmol OSO_3^-/m^2
Δ-Δ PL.HBr/AEROSIL σ_o = 0.04 µmol $Si-O^-/m^2$ *
* in the absence of PL.HBr.

In fig. 5.1 adsorption isotherms of PL.HBr-L DP 192 on PS (latex M_3) and AEROSIL OX50 pyrogenic silica are plotted. The adsorption has a high affinity character, due to the relatively high DP of the PL.HBr. So up to the plateau value virtually all added polylysine is bound to the PS or silica particles. Increasing the PL concentration after reaching the plateau value does not change the composition of the complex. At the beginning of the plateau the PL.HBr/PS complex is positively charged because the ratio $\rho_{max} = [NH_3^+]/[OSO_3^-]$ is then 2.1, where $[NH_3^+]$ and $[OSO_3^-]$ stand for the total concentration NH_3^+ and OSO_3^- groups in the system respectively. Below or at Γ_{max}, $[NH_3^+]$ and $[OSO_3^-]$ correspond also to the number of NH_3^+ and OSO_3^- groups per unit area respectively. For latex M_4 a ratio ρ_{max} of 2.8 was found. The adsorption of PL.HBr on PS is also superequivalent ($\rho_{max}>1$) in the plateau and the same is the case for the PL.HBr-silica system.

A similar behaviour was found by Philipp for the complex formation between a strongly branched polyethyleneimine with anionic carboxymethylcellulose and ligninsulphate (*Philipp, 1982 p.5*). The physical reason for the formation of non stoichrometric complexes is mainly that it is sterically not possible that every polycation charge neutralizes locally a polyanion charge or visa versa. When the polycation and polyanion are both linear flexible polyelectrolyte molecules and other attractive forces are of minor importance, no sterical constraints for 1:1 complex formation are present and 1:1 stoichiometry is observed indeed. In general the electrokinetic charge of a polyelectrolyte complex will be lower than the charge calculated from the composition of the complex due to co-adsorption of micro-ions.

As has been shown in the previous chapter there is no molecular mass dependence of the plateau adsorption at low ionic strength values. A large part of the PL.HBr residues is then in the close vicinity of the particle surface. Of course the number of PL molecules bound per PS or silica particle is strongly dependent on the degree of polymerization and the dimensions of the colloidal particles.
The influence of particle curvature (i.e. size) on the amount of polycation adsorbed per unit area is only minor as was shown by *Eggert (1976)*. This was concluded form adsorption measurements of poly (1,2-dimethyl-5-vinylpyridinium) bromide on negatively charged polystyrene particles of various sizes (100-1100 nm).

An important difference between the silica and polystyrene substrates discussed in chapter 4, is the ionic strength dependence of the

amount of PL.HBr adsorbed. In the PL.HBr/PS system the amount adsorbed is increasing up to at least 1.0 M NaBr, showing the importance of hydrophobic interactions in this case. However at constant pH the amount adsorbed on silica is decreasing with c_{NaBr} above 0.01 M NaBr. The non-coulombic attraction energy per PL segment (probably H-bonds) is only weak in this case.

5.3.2 The PS latex - PL.HBr system

Conductometric studies

Fig. 5.2 shows the variation of specific conductivity when PL.HBr-L is progressively added to PS latex M_4 (4.2 µmol $(OSO_3^-...H^+).kg^{-1}$) in the presence of H^+ counterions only. Also the addition of PL.HBr to positively charged latex (10 $kg.m^{-3}$) is shown. The curves for the

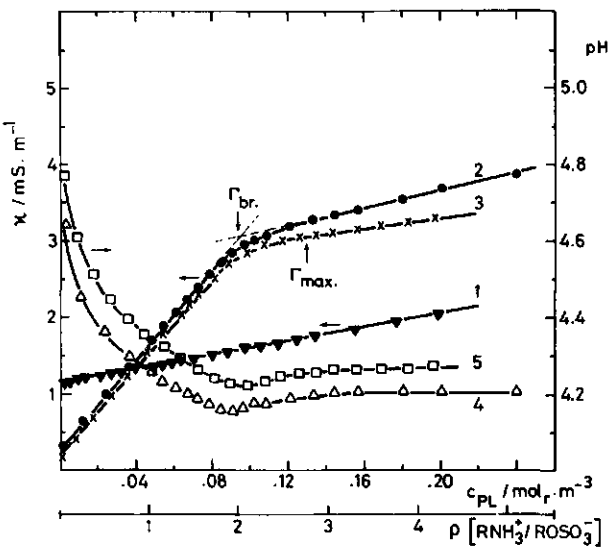


Fig. 5.2 Variation of the conductivity and pH when a solution of PL.HBr-L (pH=6; α=0) is added to diluted latex M_4 (4.2 mmol $-OSO_3^-.H^+$/kg) or latex P(+) (~10 $kg.m^{-3}$) T = 293.15 K
1. PL.HBr-L (DP 1683)/latex P(+) ; κ vs c_{PL}; 2. PL.HBr-L (DP 192)/latex M_4, κ vs c_{PL}; 3. PL.HBr-L (DP 1683)/latex M_4, κ vs c_{PL}; 4. PL.HBr-L (DP 192)/ latex M_4, pH vs c_{PL}; 5. PL.HBr-L (DP 1683)/latex M_4, pH vs c_{PL}.

negatively charged latex show a break in slope, while for the positively charged latex a completely straight line is observed. The position of the break is, as expected for flat adsorption, independent of the molecular mass of the PL.HBr used. Because the breakpoint lies at a total PL.HBr concentration where the plateau adsorption (Γ_{max} = 1.15 $\mu mol.m^{-2}$) is not yet reached, all PL.HBr added up to this point is bound to the PS particles. At the breakpoint in the conductometric titration curve the ratio ρ_{br} between the total number of $R\text{-}NH_3^+$ and $R\text{-}OSO_3^-$ groups present gives the composition of the complex. Its value is 2.0, thus also the breakpoint in the conductometric titration reflects the non stoichrometric superequivalent behaviour of the complex formation. The straight line observed with positively charged latex shows, as expected, the absence of a complex in this case, nor could any adsorption be detected analytically under these conditions. As opposed to the complex formation described above, PL.HBr and dissolved polystyrene sulphate (PSS) form a stoichrometric 1:1 complex as is shown in fig. 5.3. Here a 1:1 complex can be formed because both polyelectrolytes involved are flexible and linear as already said before.

Another difference between the PL.HBr/H.PSS and PL.HBr/PS (latex) systems is that the distance between the $-OSO_3^-$ groups on the PSS^- chain and beteen the $-NH_3^+$ charges on PL.HBr (α=o) are not very apart, 0.25 nm

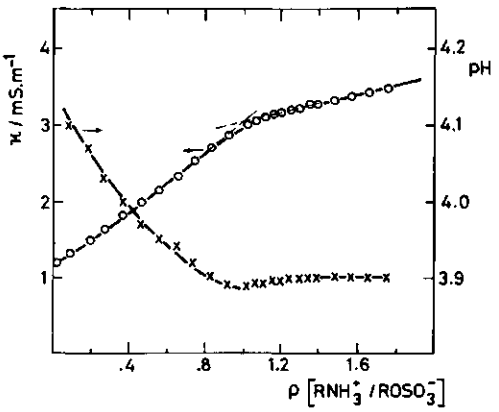


Fig. 5.3 Variation of the conductivity and pH when a solution of PL.HBr-L (DP 192); pH~6 is added to a HPSS solution (0.1 $mol_r.m^{-3}$; M(PSSH) = 88000) ·—· κ vs ρ; x——x pH vs ρ; T = 293.15 K.

and 0.36 nm respectively, whereas the mean distance between the $-OSO_3^-$ groups on the PS particles is 2 nm. When the polyanion and polycation are both linear and entirely flexible it is not necessary for the formation of a 1:1 complex that the distance between the charges on each chain is about equal. The structure of the complex formed is however dependent on these distances. When the distances on both chains are about equal a ladder-like structure can be formed (*Tsuchida et al., 1974; Philipp, 1982*), while if these distances are not equal a more random structure is likely (*Philipp, 1982*). Because the $-OSO_3^-$ groups on the PS (latex) surface are at an average distance of 2 nm, it is sterically impossible to accommodate one $-NH_3^+$ onto each $-OSO_3^-$ group, also when the $-OSO_3^-$ groups are slightly movable because they are bound to chains protruding into the solution, so called hairs (see section 3.2.3). In the terminology used by *Polderman (1975)* the PL.HBr/PSS system is a symmetric system while the PL.HBr/PS (latex) system is asymmetric.

The slope of the κ vs c_{PL} curves, i.e. the molar conductivity Λ after the breakpoint must be identical to that of dissolved PL.HBr (when double layer effects may be neglected). This is shown by the equality of the slope of curve 1 after ρ_{br} and the slope of bulk PL.HBr (curve 3) in fig. 5.2. Before the breakpoint the slope is much higher. This is caused by the release of microions, due to the binding of $-NH_3^+$ groups of lysine to the sulphate groups on the PS surface:

$$R\text{-}OSO_3^- \ldots H^+ + R\text{-}NH_3^+ \ldots Br^- \rightleftarrows R\text{-}OSO_3^- \ldots NH_3^+\text{-}R + H^+ + Br^- \qquad (1)$$

The same process occurs also in the PL.HBr/PSS^-H^+ system. That H^+ and Br^- is released due to the complex formation is also shown by the decrease in pH occurring. When all $-OSO_3^-$ groups are bound to an $-NH_3^+$ group no H^+ is released anymore and the pH reaches a minimum value. This pH minimum coincides with the breakpoint in the κ vs c_{PL} curves. Λ_{HBr}(293.15 K) found from the κ vs c_x curves, 29.5 $mS.m^2.eq.^{-1}$, is about 20-25% lower than Λ_{HBr}(293.15 K; 0.1 $mol.m^{-3}$). The lower molar conductivity of HBr can be due to the lower activity of the ions in the electrical double layer of the PS particles and polylysine (*Manning, 1972*). When however the amount of released protons per kg PS particles at the conductivity breakpoint is calculated from the measured pH difference neglecting activity coefficients and the suspension effect, a value of 4.09 ± 0.3 $\mu mol\ H^+.kg^{-1}$ is found. This value is close to the value of the surface concentration $-OSO_3^-$ found for this

latex from conductometric proton titrations. So the binding of Br^- to the peptide group of PL (*Ciferri et al., 1968*) can also contribute to the lower Λ_{HBr} found.

Another point is that no break is observed in the conductivity curves at ρ=1. This shows that the charge stoichiometry of the complex formation does not change up to ρ_{br}. At ρ=1 when the particles are overall neutral the latex is flocculated, because no overall repulsive interaction between the particles is present anymore (*Ho and Howard, 1982; Gregory, 1973; Kasper, 1971*). As pointed out by *Kasper (1971)* and also by *Gregory (1973)* positively charged and negatively charged patches exist on the PS particles surface at ρ=1. The conductometric titration curves found here support this model. Visual flocculation of the latex-PL.HBr system during the titration experiments was always observed above ρ=1 but well below the breakpoint.

In homogeneous polycation-polyanion systems the entropy increase due to the release of microions is usually the driving force for the complex formation (*Philipp, 1982; Manning, 1978; Record, 1978*). This is probably also an important factor in the PL.HBr/latex system used here. Preliminary microcalorimetric measurements performed by us on the PL.HBr latex system at pH 6 showed a clearly exotherme heat of adsorption. Thus also enthalpic interactions favour the adsorption. *Ross and Shapiro (1974)* found the binding of polylysine to DNA to be almost athermal, so in this case the ion release is the sole driving force.

In fig. 5.2 one can see that the amount PL.HBr adsorbed in the breakpoint of the conductometric titration curve Γ_{br} is 0.84 $\mu mol.m^{-2}$ is not identical with the maximal adsorption (Γ_{max} = 1.2 $\mu mol.m^{-2}$) but lower. This shows that after the complete neutralization of the $-OSO_3^-$ groups at Γ_{br}, the adsorption still increases. This can only be the case if other than electrostatic interactions contribute to the interaction free energy between a PL segment and the PS surface. As was also concluded from the salt dependence of the adsorbed amount, hydrophobic interactions between the hydrophobic PS and the $-(CH_2)-_4$group of a lysine residue side chain are probably responsible for this.

Direct estimation of the fraction PL charges not bound to an $-OSO_3^-$ group

An interesting experiment was the direct measurement of the free $R-NH_3^+$ lysine groups in adsorbed poly-L-lysine. To this end dilute PS

latices (11.25 $kg.m^{-3}$) were first titrated with PL.HBr-L (DP1683), one sample till just below Γ_{br} (c^T_{PL}=0.0806 $mol_r.m^{-3}$) and one till above Γ_{br} but below Γ_{max} (c^T_{PL}=0.0983 $mol_r.m^{-3}$). Then the latex with adsorbed PL.HBr was titrated with a PVS·K solution. Blanc titrations of water and a PL.HBr solution with PVS.K were performed to serve as reference titrations.

In fig. 5.4 conductivity curves for these various titrations are collected. Competitive polyelectrolyte adsorption plays a role in the titrations of adsorbed PL with PVS.K. This explains the rather bended shape of the curves. The titration curves of adsorbed PL.HBr with PVS.K show two breaks. The first reflects the disappearance of free

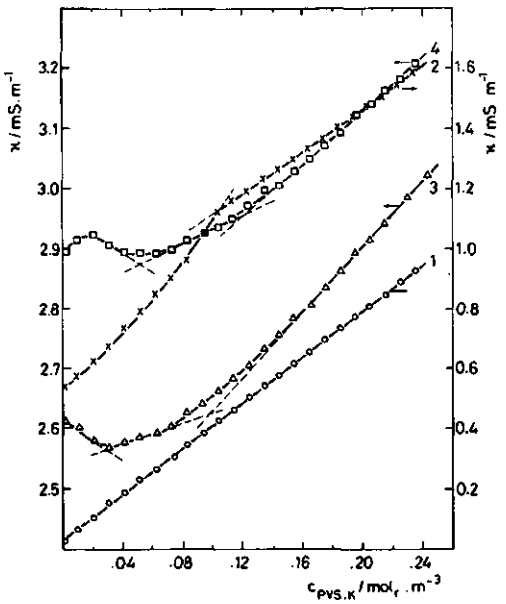


Fig. 5.4 Variation of the conductivity when a solution of potassium polyvinylsulphate (10.5 $mol_r.m^{-3}$) is added to

1. 20.0 cm^3 water initial pH = 5.8;
2. 20.18 cm^3 PL.HBr-L (DP 1683) c^T_{PL} = 0.0806 $mol_r.m^{-3}$ initial pH 5.02
3. 20.18 cm^3 latex M_4 (11.25 $kg.m^{-3}$) with adsorbed PL-HBr-L (DP 1683) c^T_{PL} = 0.0806 $mol_r.m^{-3}$ initial pH 4.24.
4. 20.22 cm^3 latex M_4 (11.25 $kg.m^{-3}$) with adsorbed PL.HBr-L (DP 1683) c^T_{PL} = 0.0983 $mol.m^{-3}$ initial pH 4.47.

in 3 and 4 PL.HBr is adsorbed quantitatively. T = 293.15 K

Notice the different κ scales.

$-NH_3^+$ groups. The occurrence and position of the second break shows that PL.HBr is displaced from the $-OSO_3^-$ groups of the polystyrene at higher PVS.K concentrations. When also in the adsorbed state stoichiometric charge complexes between the free $R-NH_3^+$ groups of PL.HBr and sulphate groups of PVS.K are found, the ratio between the first break and second break in the κ vs $c_{PVS.K}$ curve is the fraction of free $-NH_3^+$ charges. This results in values of 0.3 and 0.4 for this ratio for adsorption values of respectively 0.72 and 0.84 $\mu mol.m^{-2}$. The increase of the fraction free charges with increasing amount PL adsorbed suggests that the fraction of the total adsorbed amount PL in loops and tails increases with increasing surface coverage. This trend is also predicted from polyelectrolyte adsorption theory (*van der Schee, 1984*).

It is also possible to calculate the fraction free $-NH_3^+$ charges from the amount PL.HBr adsorbed and the surface concentration $-OSO_3^-$ groups. For the adsorption values mentioned above: 0.72 and 0.84 $\mu mol.m^{-2}$, values for the fraction free $-NH_3^+$ charges of 0.4 and 0.5 are found. The difference between these values and those found from the titrations with PVS.K are probably the consequence of the uncertainty in the value of the surface charge of the PS particles. Another possibility is a deviation from the 1:1 complex formation between adsorbed PL.HBr and PVS.K, because of the rather flat conformation of the adsorbed PL molecules.

A similar type of experiment as described above was suggested by *Eisenlower (1982)* and performed by *Horn (1978)* with the polyethyleneimine/polystyrene latex system. However Horn used a metachromatic cationic dye for the optical detection of the free polyanion concentration. At the adsorption plateau Horn found a value of 0.4 for the fraction free charges of the adsorbed polyethyleneimine (PEI). In this case no displacement of the polycation by PVS.K could be detected. In both adsorbed PL.HBr and PEI the rather low fraction free charges found is the consequence of the flat conformation of adsorbed polycation molecules.

In fig. 5.5 the pH variations measured upon addition of PVS.K to PL in solution or adsorbed are plotted for the same titrations as given in fig. 5.4. A pH increase up to the conductometric breakpoint is observed when PVS.K is added to a PL.HBr solution, while addition of PVS.K to adsorbed PL.HBr results in a pH decrease over the whole concentration range measured. The effect is too large to be accounted for only from changes in the proton activity during the titration with PVS.K.

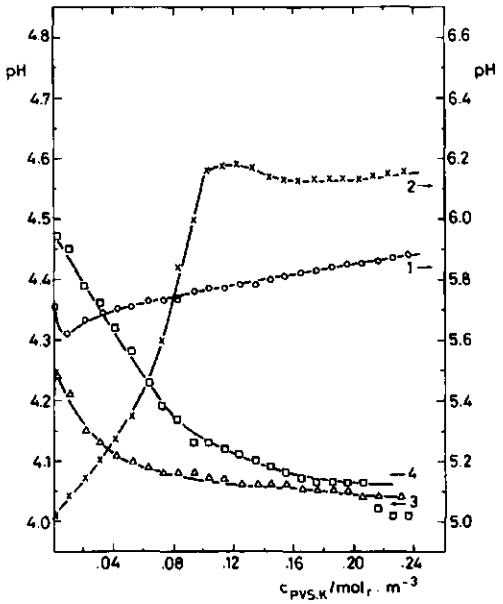


Fig. 5.5 Variation of the pH when a solution of potassium vinylsulphate (10.5 mol$_r$ m^{-3}) is added to water (1), a PL.HBr solution (2) or PL.HBr adsorbed on PS latex (3 and 4). See the legend of fig. 5.4 for further details.
T = 293.15 K.
Notice the different pH scales.

Conductometric titrations at high pH

The complex formation between PL.HBr and PS particles was also investigated at pH 10.2. The degree of dissociation of the PL $-NH_3^+$ groups is then about 0.6-0.7. In fig. 5.6 the results of the high pH titrations are shown. Also here breaks in the κ vs c_{PL} curves are observed. The slope of the κ vs c_{PL} curves after the break is again identical to that of aqueous PL.HBr at a pH of 10.2. The amount PL.HBr adsorbed in the breakpoint is again independent of the molecular mass of the PL, but higher than that at low pH value, viz. 1.33 μmol.m^{-2} and 1.24 μmol. m^{-2} for PL.HBr (DP 19; α=0.64) and PL.HBr (DP 1683; α=0.70) respectively. When these adsorption values are multiplied by the degree of dissociation α, the adsorption values Γ_{br} at pH 4.2 are obtained in both cases. This shows that also at high pH, i.e. at lower PL chain charge density,

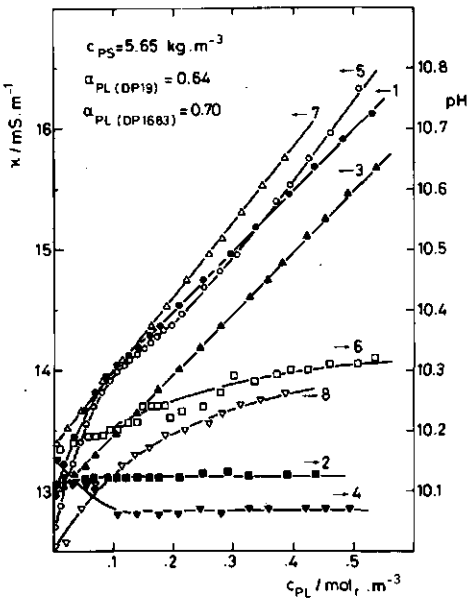


Fig. 5.6 Variation of the conductivity and pH when a solution of PL.HBr-L (initial PH 10.2) is added to diluted PS latex M_4 (5.65 kg.m^{-3}; 4.2 mmol ($-OSO_3^-$).kg^{-1}; initial pH 10.2) or water (initial pH 10.1 ± 0.1) T = 293.15 K.
Addition of PL.HBr to latex M_4: 1. and 5.: κ vs c_{PL}; 2. and 6.: pH vs c_{PL}
Addition of PL.HBr to water: 3. and 7.: κ vs c_{PL}; 4. and 8.: pH vs c_{PL}
Open symbols: PL.HBr-L (DP 19); Filled symbols: PL.HBr-L (DP 1683).

the non charge stoichiometry of the complexation is the same as it is at α=0 and that all $-OSO_3^-$ groups on the PS particles are bound to an $-NH_3^+$ group of the polycation. Because at pH 10.2 Γ_{br} is higher than at pH 4.2 the fraction of PL segments in loops and tails is higher at pH 10.2. The interaction of the poly-L-lysine with polystyrene is analogous to the complex formation between polymethacrylic acid and polyammonium polymers (charges in the chain backbone) investigated by *Tsuchida et al. (1974)*.

The plateau adsorption value found for the adsorption of PL.HBr-L (DP 192) from water pH 10.2 on PS is 4.8 μmol.m^{-2}. So Γ_{max} is much higher than Γ_{br} at high pH. Non electrostatic contributions to the adsorption energy per segment are now relatively more important than at low pH. In other words the lateral repulsion between the $-NH_3^+$ groups in loops and tails of adsorbed polylysine molecules is strongly

reduced due to the lower chain charge density and consequently of less importance (see also section 6.5.3).

5.3.3 The silica-polylysine system

The silica-polylysine.HBr system is more complicated than the latex-PL.HBr system because the surface silanol groups on the silica particles have a weakly acidic character. The dissociation of these groups is, as will be shown later, strongly influenced by the complexation with polylysine. Adsorption isotherms of PL on silica show the same general features as those on polystyrene (see section 5.1). However the adsorbed amount is now also strongly dependent on the pH in the acidic region.

In fig. 5.7 the variation of the conductivity and pH when a solution of poly-L-lysine.HBr (DP 192) is added to a silica sol is plotted against the total PL concentration in the system.
Curves 1 and 3 show the results for an initial pH of 5.50. Here the counterions of the AEROSIL are only H^+. The curves are very similar to those found for PS latex as a substrate for PL. The slope after the break is again the same as that for PL.HBr in water. Before the break the higher slope is caused by the release of H^+ and Br^- ions when the complex is formed and about identical to that found for the PL/PS latex system. The release of acid is also shown by the decreasing pH with increasing c_{PL} up to the conductometric breakpoint, after which the pH remains constant. During the addition of PL.HBr the degree of dissociation of the silanol groups increases, because PL act as specifically adsorbing counterions.

At the breakpoint the adsorbed amount is such that the maximal amount of $Si\text{-}O^-$ groups are bound to $-NH_3^+$ groups of lysine. After the break the adsorption still increases as is evident from depletion measurements, but the degree of dissociation of silanol groups does not change anymore, because otherwise the pH after the breakpoints in fig. 5.7 would still be changing. The increase in adsorption after the conductometric breakpoint is possible because the likely formation of hydrogen bonds between undissociated silanol groups and peptide units of the PL. Also dipolar interactions may play a role.

The formation of H-bonds and other interactions are not important in the otherwise comparable homogeneous system polyglutamic acid/poly-L-lysine investigated by *Domard and Rinaudo (1980, 1981)*. Here always stoichiometric complexes are found such that all $-NH_3^+$ groups are bound

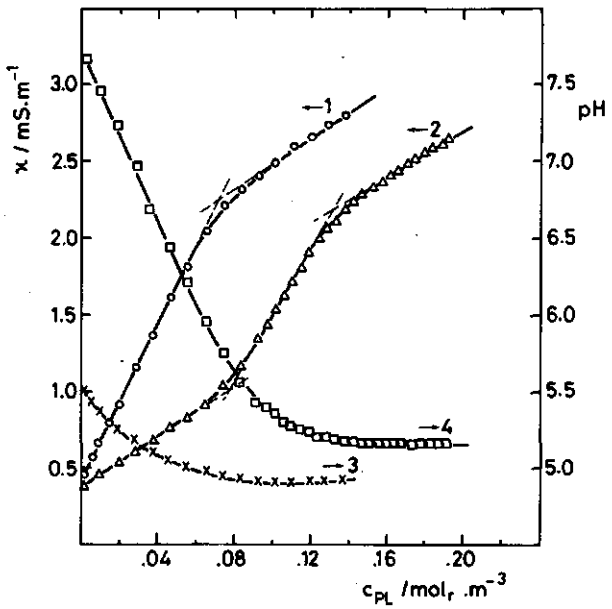


Fig. 5.7 Variation of the conductivity and pH when a solution of PL.HBr-L (DP 192) is added to an AEROSIL OX50 sol (12.65 kg.m^{-3}). T = 293.15 K. 1. κ vs c_{PL} initial pH = 5.50, 2. κ vs c_{PL} initial pH = 7.66; 3. pH vs c_{PL} initial pH = 5.50; 4. pH vs c_{PL} initial pH = 7.66.

to an $-COO^-$ groups of polyglutamic acid. No interaction takes place with an undissociated carboxylic group.

At a starting pH of 7.66 (curves 2 and 4 of fig. 5.7) Na^+ ions are the dominant counterions of silica but the silica is still incompletely charged. The conductivity titration curve shows now two breaks. After the second one the slope is the same as that after the break for curve 1. At the second break the breakpoint adsorption is thus maximal again and the compensation of surface $\equiv Si-O^-$ groups maximal. The maximal breakpoint adsorption is again lower than Γ_{max}.
The slope of the conductivity curve 2 before the first break is lower than that after the break, because initially Na^+ and Br^- rather than H^+ and Br^- are the released microions. The pH is decreasing because due to the PL.HBr binding the dissociation of the silanol groups increases, i.e. the apparent pK of the silanol groups decreases because of the strong possitive field of the PL molecules. While the pH is decreasing

all Na^+ is displaced and further H^+ is becoming to compete with Na^+ and an increase in slope of the κ vs c_{PL} curve is observed. Thus after the first break in curve 2 mainly H^+ and Br^- ions are released just as was the case at an initial pH of 5.5.

A point of discussion is whether the released protons are originating from the silanol groups or the NH_3^+ groups of PL. The latter is unlikely because in the PL.HBr/latex system no groups are titrated below pH 7 also not when PL is adsorbed (see chapter 6). This is because the intrinsic pK of the NH_3^+ groups is about 10.8. The specific conductivity at the second break in curve 2 (fig. 5.7) and the break in curve 1 (fig. 3.9) are about equal. So the maximal amount of released micro-ions is not dependent on the initial pH of the silica sol. To find out whether the PL-silica complex at the breakpoint are charge stoichiometric or not, the surface charge density of the silica in the presence of PL must be known. A conductometric proton titration with NaOH (0.1 M) from pH 4.9 to 8 after a titration of a silica sol with PL.HBr yielded a $-Si-O^-$ surface concentration of 9.88 $\mu mol.g^{-1}$, while at the same pH the surface concentration in the absence of PL, obtained by the same method, is only 2 $\mu mol.g^{-1}$. The ratio ρ_{br} (pH 4.9) is then 0.6, i.e. smaller than 1. The complex is non charge stoichiometric but not yet superequivalent with respect to the adsorbed amount PL at Γ_{br}, essentially because the charge is not the origin of the adsorption but the cause. At the maximal adsorption at pH 4.9, determined analytically, the value of ρ is 2.88. Then clearly a superequivalent complex is present (see also section 5.3.4). Because of the high silanol group density the possibility to form a 1:1 complex is greater than in the case of latex.

5.3.4 Influence of the surface charge density and ionic strength on the polylysine-charged particle interaction

As already discussed in section 4.3.6, the amount of PL adsorbed on polystyrene increases with increasing salt concentration.
A conductometric investigation of this salt effect is limited to fairly low salt concentrations because otherwise the variations in conductance due to the binding of PL are outweighted by the swamping electrolyte added.

In fig. 5.8 the conductometric titration of latex M_4 with PL.HBr (*pH = 6; α = o*) in the presence of 10^{-3} *M* NaBr is shown. It is found that the breakpoint is much more rounded than in the absence of salt.

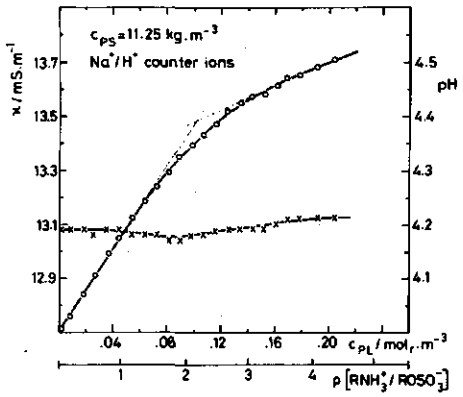


Fig. 5.8 Variation of the conductivity and pH; when a solution of PL.HBr-L (pH = 6; α = o) is added to diluted latex M_4 (4.2 μmol ($-OSO_3^-.H^+$) kg^{-1}, in the presence of 10^{-3}M NaBr. T = 293.15 K o—o κ vs c_{PL}; x—x pH vs c_{PL}.

Extrapolation of the linear parts of the curve yielded a slightly higher (0.89 μmol.m^{-2}) 'breakpoint' adsorption than in the absence of low molecular mass electrolyte: Γ_{br} = 0.84 μmol.m^{-2}. The difference found is probably within experimental error. *Michaels et al. (1965)* found deviations from the 1:1 complexation between polyvinylbenzyltrimethylammonium chloride and polystyrene sulphonate only above 0.1 M added NaCl. In my view, above 0.1 M electrolyte, charge interactions are screened to such an extend that hydrophobic interactions can become dominant and govern the complex formation. This results then in a complex with a charge stoichiometry, which differs significantly from that at low salt concentration. When such interactions are absent as is the case in the polylysine -DNA complex, dissociation of the complex at higher salt concentration occurs (*Manning, 1978*).

At high salt concentrtaions i.e. > 0.1 M also sterical factors contribute to the stoichiometry of the complex formation (*Michaels et al., 1965*). Due to the stronger coiling of the polyions at high salt concentration not all ionic sites are accessible to sites of the oppositely charged polyion. The stoichiometry of the complex formation deviates therefore from the low or no added electrolyte situation (*Michaels et al., 1965*). The stronger coiling of the polyions at high ionic strength is especially important when non equilibrium states are involved in

the complexation reaction. However the occurrence of non equilibrium states is difficult to trace for homogeneous as well as heterogeneous polycation-polyanion systems.

A clear difference between the PL/latex system in the presence of 10^{-3} M NaBr or in the absence of it is the pH variation when PL.HBr is added. At 10^{-3} M NaBr no decrease in pH is observed on addition of PL.HBr. Due to the presence of NaBr the diluted latex is in the Na^+ form. Binding of PL.HBr causes now the release of Na^+ and Br^- instead of H^+ and Br^-, a process without any pH effect occurring.
In fig. 5.9 the conductometric breakpoint adsorption found from the variation in conductance, when poly-L-lysine.HBr (DP=192; pH=6) is added to silica sols at various salt concentrations, is shown. The pH at the breakpoints is also indicated. Just as is found from depletion measurement (see section 4.3.6), the amount adsorbed Γ_{br} increases with increasing ionic strength in the studied range. In contrast with the behaviour of the PL/latex system in the presence of NaBr, the pH is decreasing when PL.HBr is added to the silica sols in the presence of NaBr. This can be explained by a further increase of the silanol group dissociation when PL.HBr binds to the silica surface.

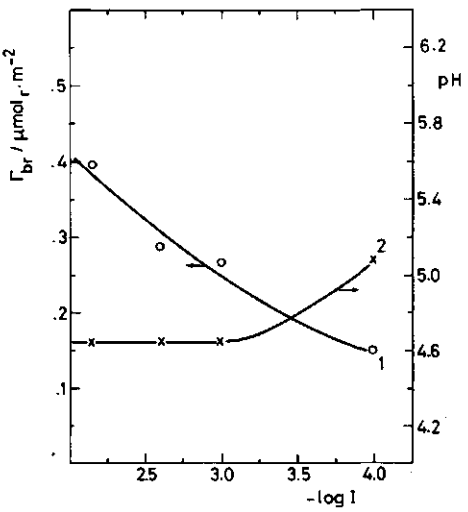


Fig. 5.9 Adsorbed PL.HBr-L (DP 192) in the conductometric breakpoint as a function of the ionic strength (curve 1). The pH values in the conductometric breakpoints are also indicated (curve 2). Substrate: silica.

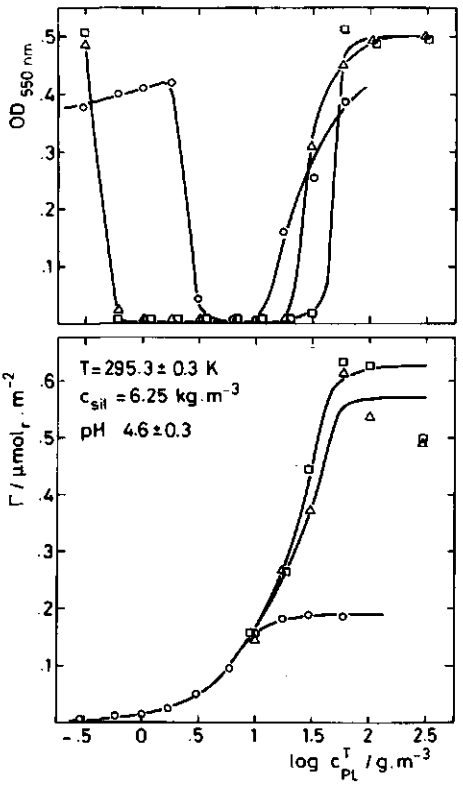


Fig. 5.10 Sol stability (upper curves) and adsorbed amount PL (lower curves) for the silica-PL.HBr system as a function of the total concentration PL.HBr (DP 192) on a log scale.
o—o No added NaBr; Δ–Δ 1.10^{-3} M NaBr; 5.10^{-3} M NaBr.

It is interesting to compare the conductometric results described above with the sol stability and simultaneously obtained adsorption curves for the silica/PL system (fig. 5.10). Note that in fig. 5.10 Γ_{max} is plotted against the logarithm of the *total* concentration PL.HBr present. The stability of the silica sols as a function of the total concentration PL.HBr i.e. as a function of surface coverage, show the same picture as generally observed for the adsorption of polycations on negatively charged particles. See for example *Ho and Howard (1982) Bleier and Goddard (1980)* and *Lindquist (1975, 1976)*. After floc-

culation of the silica by low adsorbed amounts PL, restabilization occurs at higher adsorption values due to charge reversal and perhaps also the occurrence of steric repulsion. The PL/silica system shows a broadening of the instability domain range when the salt concentration is increased. The principal mechanism for flocculation is thought to be charge neutralization due to the PL adsorption. Total neutralization is however not required for maximal sedimentation. This is one of the observations leading to the picture of the existence of electrostatic patches on the particle surface (*Bleier and Goddard, 1980*). Because of the long incubation times of the flocculation experiments, the optimal flocculation concentration was only observable in the absence of added NaBr. When it is assumed that the isoelectric concentration corresponds to the c^{T}_{PL} value where the steep rise of the stability curves starts, the corresponding adsorption values (Γ_{iso}) coincide rather well with Γ_{br}, the breakpoint adsorption (see Table 5.1).

Table 5.1 Adsorption values of poly-L-lysine.HBr (DP=190) at AEROSIL 0X50 at pH 4.60 for various electrolyte concentrations.

c_{NaBr} M	Γ_{br} ($\frac{\mu mol}{m^2}$)	Γ_{iso} ($\frac{\mu mol}{m^2}$)	Γ_{max} ($\frac{\mu mol}{m^2}$)	σ_o ($\frac{\mu mol}{m^2}$)	ρ_{br}
$< 10^{-4}$	0.11	0.15 ± 0.03	0.2 ± 0.05	0.20*	0.6
1.10^{-3}	0.27	0.25 ± 0.04	0.6 ± 0.1	0.29	0.9
5.10^{-3}	0.40	0.45 ± 0.06	0.6 ± 0.1	0.22+	1.8

* obtained from a conductometric proton titration of silica after a titration with PL.HBr.

\+ Interpolation of the σ_o values of silica in the presence of excess PL at pH 4.60 between 10^{-3} and 10^{-2} M NaBr respectively

The values of Γ_{iso} may be considerably in error because of the uncertainty in the iso-electric PL concentration, however the correspondence between Γ_{br} and Γ_{iso} suggests that in the silica/PL system the conductometric breakpoint occurs at about isoelectric adsorption. When no specific adsorption of microions takes place a value of 1.0 would have been found for ρ_{br} at the ionic strengths investigated. However this seems contradictory with the increase found in ρ_{br} with increasing ionic strength, when ρ_{br} is calculated from Γ_{br} and σ_o. More experiments are needed to interpret these trends further. Especially micro electrophoresis could give additional information about

the sign and magnitude of the charge of PL coated particles at Γ_{br}.

In fig. 5.11 the maximum adsorption obtained from depletion measurements in the absence of NaBr and the breakpoint adsorption Γ_{br} are plotted against the equilibrium pH. The increase of Γ_{max} is caused by the increase in σ_o with pH. Here also the effect of PL.HBr on the surface charge becomes visible, because in the absence of PL the increase of the surface charge with increasing pH is hardly measurable in the absence of salt.
Between pH 4.2 and 4.8 Γ_{max} is higher than Γ_{br}. As stated before hydrogen bonding between PL peptide units and silanol groups is probably responsible for this. It is remarkable that Γ_{br} remains constant between pH 4.2 and pH 4.8, whereas σ_o increases with increasing pH. This shows that the charge stoichiometry at Γ_{br} changes with increasing pH, towards lower ρ_{br} values. This and the observation that ρ_{br} at pH 4.6 is smaller than 1.0 suggests that the amount for NH_3^+ groups unaccessible $Si\text{-}O^-$ groups increases with increasing pH. The gellayer on the silica surface, though small (see *Yates and Healy, 1976*) must be responsible for this.

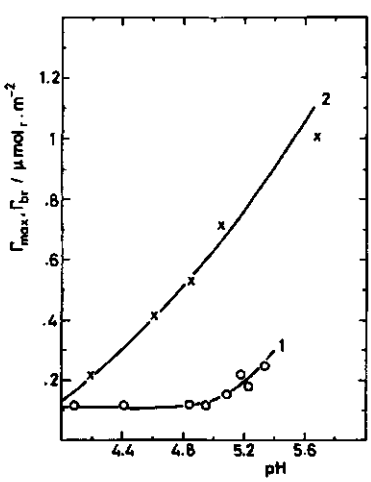


Fig. 5.11 Comparison of the amount PL.HBr-L adsorbed in the breakpoint (curve 1) and the analytical adsorption (curve 2) as a function of the equilibrium pH. No added NaBr present.

5.3.5 Proton titrations of silica covered with PL

In fig. 5.12 two sets of charge-pH curves of AEROSIL 0X50 are shown. Set one comprises the titration curves of bare silica at various electrolyte concentrations. Set 2 represents those in the presence of so much excess PL.HBr, as to ensure plateau adsorption at each pH.

The p.z.c. of bare silica is taken at pH 3.0, following *Sonntag (1976, 1980)* and *Abendroth (1970)*. Because of the flat charge-pH curves found in the region around the p.z.c., the exact position of this point does not greatly influence the position of the charge-pH curves. In the presence of PL.HBr also a value of 3.0 was taken for the p.z.c., although the p.z.c. is probably shifted to lower pH, i.e. in a more positive direction. Such a shift is expected theoretically and observed experimentally in the polylysine-AgI system investigated by *Van der Schee (1982)*. The flat titration curves of silica make a detection of a p.z.c. shift in the silica-PL system impossible. It is also because of this flatness that the results are insensitive to the actual choice.

The set of curves found for bare silica agree well with those found by *Sonntag (1976, 1980)* for AEROSIL OX50 in KCl solutions. Quantitative differences may be attributed to the different silica batches used. Also the kind of micro 1:1 electrolyt used gives rise to small differences (*Abendroth, 1970*). The behaviour found is typical for oxides. The dissociation of the silanol groups increases with increasing pH resulting in higher surface charge densities at higher pH. Increase of the electrolyte concentration at constant pH results also in an increase of the surface charge. This is caused by the screening of the $Si\text{-}O^-$ charges, which again results in an increase of the silanol group dissociation. In other words, the apparent pK of the silanol groups decreases when the salt concentration is increased.

The proton titration curves of AEROSIL OX50 change drastically in the presence of PL.HBr. This was already expected from the conductometric results. As explained before, up to a pH of ~8, the $-NH_3^+$ groups of PL.HBr are not titrated because of the high pK_o value of these groups: 10.8. In other words under the conditions chosen, PL acts as a strong, multivalent cation. The titration curves of silica in the presence of PL.HBr show two main effects both caused by the presence of the polycation. In the first place the surface charge, i.e. dissociation of silanol groups, increases drastically when PL.HBr is added to the silica sol. Qualitatively this can be explained as follows. Due to

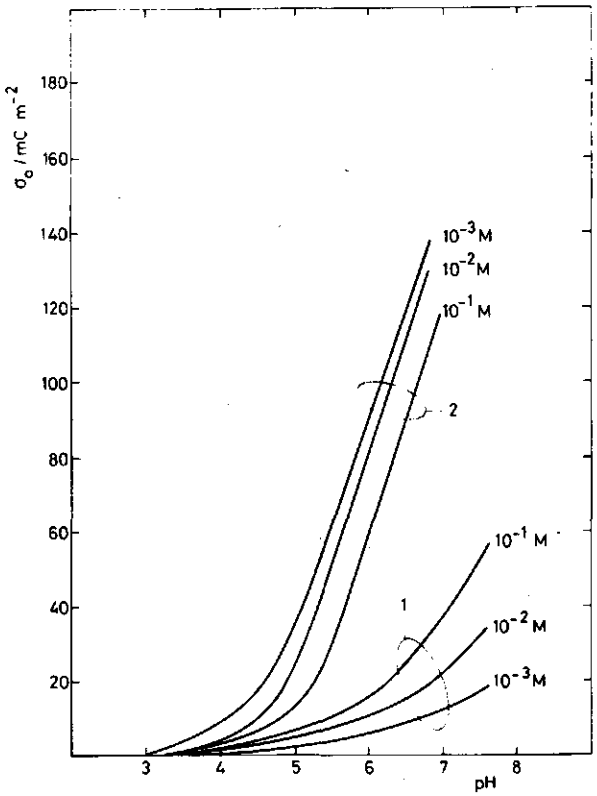


Fig. 5.12 Charge-pH curves for AEROSIL OX50 silica.
1. No added PL.HBr-L; 2. Excess PL.HBr-L (DP 190).
A_{sp} (silica) = 60 $m^2.g^{-1}$; Electrolyte: NaBr; T = 293.15 K.

the adsorption of PL.HBr the silica particles obtain a positive envelope facilitating the dissociation of silanol groups. The consequence is the higher surface charge density observed. In the second place, the salt concentration effect is reversed as compared with bare silica. This reversal was also found from the pH changes observed, when a silica sol with adsorbed PL.HBr was titrated with electrolyte solution at various initial pH values. The effect of the reversal is caused by the competition between Na^+ ions and $-NH_3^+$ lysine groups for surface sites, which gives rise to a decrease in adsorption of PL.HBr (constant pH) at electrolyte concentrations above 0.01 M as discussed in section 4.3.6. The decrease in screening power of PL results again in an

increase of the apparent pK value of the silanol groups, and thus a decreased surface charge density with increasing salt concentration. The reversed salt effect is not due to the suspension effect, as in the presence of positively charged particles the measured pH values are overestimated at low salt concentrations due to the suspension effect. At 0.1 M NaBr the suspension effect is vanishing low (see also section 6.6.2).

The observed trends are also clearly visualized by plotting the apparent pK of the silanol groups against the degree of dissociation of these groups (fig. 5.13). A maximal silanol group surface density of 4.4 nm^{-2} is taken (*Sonntag, 1980*). Following *James & Parks (1982)*, instead of α, ($10\alpha + \sqrt{c_s}$) is plotted in order to enable extrapolation of the plots to zero α and zero ionic strength. In doing so it is implicitly assumed that all silanol groups have the same intrinsic pK value, otherwise pK_{app} has no clear meaning. When the plots of bare silica (fig. 5.13a) and those of silica in the presence of PL.HBr (fig. 5.13b) are compared the following can be observed: The polyelectrolyte character of the silica particles is reduced by PL.HBr, as judged by the decrease of the slopes of the pK vs $10\alpha + \sqrt{c_s}$ plots, and because these slopes are less dependent on the ionic strength when PL.HBr is adsorbed. Both effects are due to the positive electric field experienced by the silanol groups when PL is present. The intrinsic pK value of the silanol groups in the presence of PL.HBr is lower: 5.4 ± 0.4, than for bare silica (6.7 ± 0.4). This means that when PL adsorbs at α = 0 the silanol groups would behave more acidic. The values found are relatively uncertain because the extrapolation is not linear (see fig. 5.13b).
James and Parks (1982) found for the intrinsic pK of pyrogenic silica (Cab.O-Sil M7) a value of 7.2 ± 0.3 by replotting the data of *Abendroth (1970)*. The agreement between this value and that found here for bare silica is within experimental error.
The extrapolation to α = 0, made in the presence of PL, has only a practical meaning when the adsorbed amount is independent from α, which is not the case. Depletion measurements show that the amount PL adsorbed below pH 3.5 is inmeasurebly low.

The proton titration behaviour of homogeneous (weakly acid) polyanion-(weakly basic) polycation complexes is quite analogous to that of the silica-polylysine system. *Polderman (1975)* conducted from potentiometric proton titrations in the polyethyleneimine/polyacrylamide-acrylic acid copolymer system that both the polyanion and polycation

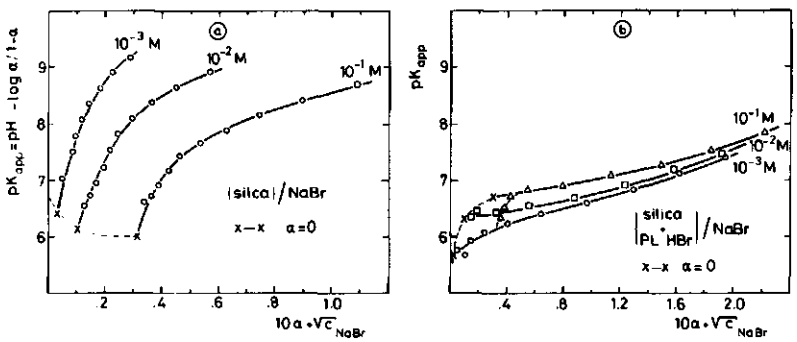


Fig. 5.13 a. Double extrapolation plot for the estimation of the intrinsic pK of surface silanol groups of AEROSIL OX50, showing the variation of the apparent pK value with fractional surface charge and NaBr electrolyte concentration. b. As fig. 5.13a but now in the presence of excess PL.HBr-L (DP 190).

in the complex behave like stronger polyelectrolytes and that the screening by the charges of the other polyion is analogous to the screening by small ions but much more effective. *Tsuchida (1974)* reached the same conclusion for the polymethacrylic acid-polyammonium polymer system. Tsuchida observed a decrease in the apparent pK of the carboxyl groups and a diminished polyelectrolyte character of PMA in the presence of the polycation. *Reinert (1981)* found a strong increase of the apparent pK (i.e. stronger basic character) of spermine (a polyamine) when complexed to polyphosphate. Reinert pointed out that the combined protonation/deprotonation and association behaviour of the spermin-polyphosphate system, may serve as a model for similar processes in biological systems. The influence of low molecular mass electrolyte upon the proton titrations of the homogeneous complexes was not investigated by the authors mentioned above.

5.3.6 Theoretical descriptions of polycation-polyanion association

Record (1978) described the interaction between polyelectrolytes and oppositely charged oligo(poly)electrolytes in homogeneous systems in terms of multiequilibrium theory for interacting sites (*Schellman, 1975*) accounting for ion condensation according to *Manning (1972)* and the usual screening effects. This theory is not useful for a quantita-

tive desription when the complexation constant is unmeasurable high as is usually the case when both polyelectrolytes have a high degree of polymerization. Besides the very high (i.e. unmeasurable high) affinity found for the adsorption of polylysine on the surface of negatively charged particles, the theory is also not applicable in this case because the appropriate statistical factors (i.e. entropy contributions) for the formation of trains, loops and tails, i.e. the structure of the adsorbed PL molecules, are not incorporated.

In stead of following this approach it it more expedient to extend a suitable theory for the adsorption of uncharged polymers with electrostatic interactions, as has been done by *Van der Schee (1984)*, with the polymer adsorption theories of *Roe (1974)* and *Scheutjens-Fleer (1979, 1980)*. The theory of Van der Schee is the only viable polyelectrolyte adsorption theory available at the moment, as will be explained in chapter 7. Provisional reports of Van der Schee's polyelectrolyt adsorption theory have also been given by *Marra et al. (1982)* and *Bonekamp et al. (1983)*.

A thermodynamic treatment of the complex formation between polyanions and polycations in homogeneous solutions (i.e. before phase separation occurs) has been presented by *Polderman (1975)*, to explain his proton titration data. This theory is only valid for homogeneous systems and in swamping electrolyte, therefore Polderman's analysis cannot be used for the proton titrations of the PL-silica system, presented here.

In chapter 7 I will discuss some aspects of the polyelectrolyte adsorption theory of van der Schee and the applicability of this theory for the description of the adsorption of charged polyaminoacids. Special attention will be paid to the effect of the salt concentration and surface charge on the adsorption properties of polylysine on AgI and polystyrene particles.

5.3.7 UV and CD spectroscopy of the silica/poly-L-histidine-system

Measurements of the UV and CD spectra of poly-L-histidine (PHis) DP 110 adsorbed at the silica water interface were performed with the following purposes.

i. Investigation of the possibilities of performing optical spectroscopy of polyaminoacids against a large background scattering.
ii. Detection of conformational differences between PHis in aqueous solution and in the adsorbed state.

Silica was chosen as the substrate for PHis because the optical pro-

perties of silica sols are most promising for performing optical spectroscopy. Poly-L-histidine was chosen because this polycation has similar adsorption properties as PL.HBr (see section 4.3.4), but, as is more important, PHis shows also, just like PL a conformation transition from a coil-like state to an ordered conformation, probably a right-handed helix on increasing the pH from 4 to 6. This was concluded from CD measurements by *Beychok (1967)*. Because of the occurrence of this transition in the acid region, silica is a suitable substrate for PHis, to study by spectroscopic means the conformation of adsorbed PHis at different pH values. At pH 10 where the helix-coil transition in PL occurs, the silica is too soluble to use it as a substrate for PL adsorption.

A major difficulty in the envisaged optical spectroscopic study, which is not usually encountered in homogeneous (bio)polymer complexes, is the high turbidity of the particle suspension or sol. This is especially so in the UV region. AEROSIL OX50 sols are relatively favourable in this respect because they are relatively transparent when compared with latex and AgI sols of comparable mass concentration. In fig. 5.14 the $OD_{10\ mm}$ (200/240 nm) is plotted against the silica concentration.

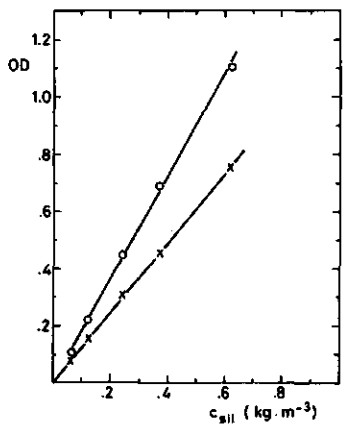


Fig. 5.14 Optical density of AEROSIL OX50 sols as a function of the sol concentration at two wavelengths. o-o 200 nm, x-x 240 nm.

One can see that at particle concentrations of 0.5 $kg.m^{-3}$ the OD is still lower than 1, which is certainly not the case with the usual latex and AgI sols. This is mainly due to the fact that the silica particles are small compared with those in latices or AgI-sols. Furthermore, the Lambert-Beer law is valid for the silica sols over the entire concentration range studied.

The measurement of an UV spectrum of an (adsorbed) chromophore present in a sol is hindered by:

(i) The presence of a large background scattering.

By taking difference spectra between a sol in the presence and absence (blanc sol) of the chromophore, corrections for background scattering can be accounted for, provided that no particle aggregation occurs.

(ii) The Duysens effect (*Duysens, 1956*), i.e. the apparent lower extinction coefficient of the adsorbed chromophore in comparence with the same chromophore in a homogeneous solution. This adsorption flattening is essentially due to the fact that part of the chromophores is not excitated by the incident beam, because they are situated in the 'shadow' of the particles present. Corrections for this effect are more difficult. Fortunately this effect is rather small when the particles are small compared with the wavelength of the incident light beam (*Duysens, 1956; Urry and Yi, 1968*) as is the case with the silica particles used here.

(iii) Multiple scattering. This effect can be ignored in the region were Beer's law applies.

In one series of experiments it was checked whether the UV difference spectrum of KHphtalate dissolved in AEROSIL OX50 sols (30.8 $mmol.m^{-3}$) of different particle concentrations was different from that of 30.8 $mmol,m^{-3}$ phtalate in water. At silica concentrations up to 0.7 $kg.m^{-3}$ the difference spectra of the non adsorbing phtalate were identical within 1% with the corresponding spectrum in water. At higher particle concentrations the phtalate spectrum becomes flattened (see fig. 5.15). Also the relative contribution of stray light from the monochromator becomes then unacceptably high. These experiments show that it is in principle possible to measure a reliable UV spectrum of a chromophore in the presence of silica, when the particle concentration does not exceed 0.7 $kg.m^{-3}$.

In fig. 5.16 the UV spectra of PHis solutions (9.09 $mmol_r.m^{-3}$) and UV difference spectra of the same solutions in the presence of AEROSIL OX50 (0.625 $kg.m^{-3}$) at pH 4.0 and 5.8 are shown. In the presence of silica the adsorbed amount exceeded 85% of the total amount

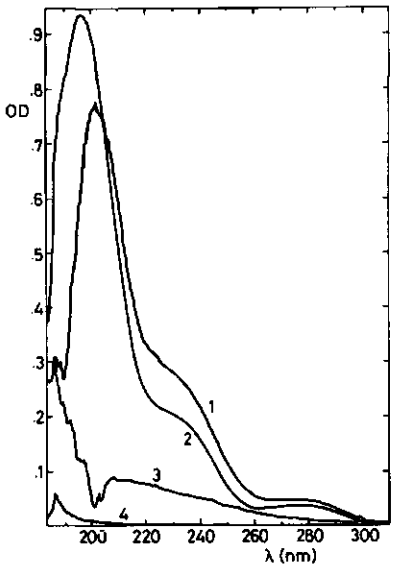


Fig. 5.15 Absorption (difference) spectra of KHphtalate ($3.08*10^{-5}$M) in the presence of AEROSIL OX50 (2.49 $kg.m^{-3}$) and in the absence of it.

1. KHphtalate (AEROSIL OX50 sol (REF.: AEROSIL OX50 sol).
2. KHphtalate in water (REF.: water).
3. Baseline for spectrum 1: both cuvettes AEROSIL OX50 sol.
4. Baseline for spectrum 2: Both cuvettes demi water.

PHis present, as indicated by the UV spectrum of the clear supernatant of the sol after centrifugation. Neither the shape of the spectrum at pH 4.0 nor that at pH 5.8 in the presence of silica was measurably different from the corresponding solution spectra. Unfortunately also no significant differences were found between the solution spectra of PHis at pH 4.0 and pH 5.8. This is probably because the imidazole ring of histidine is not part of the polyaminoacid backbone. Another difficulty is the occurrence of a baseline shift of the silica in the presence of PHis. Probably incipient flocculation in the PHis-silica system is responsible for this. Therefore no conclusions can be drawn about extinction coefficient differences between adsorbed PHis and PHis in solution.

CD measurements were performed with a single beam apparatus. Direct corrections for the silica background scattering were therefore not possible. At the maximum silica concentration which could be used

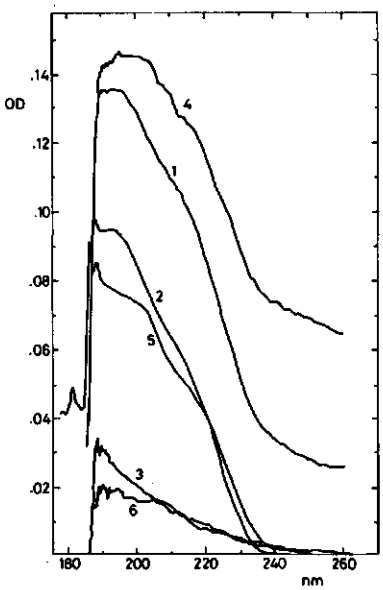


Fig. 5.16 UV absorption (difference) spectra of PHis (DP 110) in aqueous solutions of pH 4.0 and pH 5.8 in the presence of AEROSIL OX50 (0.625 $kg.m^{-3}$) or in the absence of it.

1. PHis (c^T = 9.09 $mmol.m^{-3}$)/AEROSIL OX50 sol pH 4.0; REF.: AEROSIL pH 4.0.
2. PHis (c^T = 9.09 $mmol.m^{-3}$) in water pH 4.0; REF.: water pH 4.0.
3. Baseline for curve 1: Both cuvettes AEROSIL OX50 sol pH 4.0.
4. As 1, but pH 5.8; 5. As 2, but pH 5.8.
6. Baseline for curve 4: Both cuvettes AEROSIL OX50 sol pH 5.8.

without running out of scale (0.625 kg m^{-3}), the maximally possible concentration of adsorbed PHis was too low by about a factor of five to detect the CD spectrum of adsorbed PHis. Another difficulty with the CD measurements on silica sols was that the baseline of the bare silica sol was not a straight horizontal line but increased progressively with decreasing wavelength towards negative ellipticity values.

Summarizing the following may be concluded:

i. The measurement of UV spectra of adsorbed polyaminoacids or other (bio)polymers with intrinsic chromophores is possible in principle. The

measurements can be improved when particle aggregation can be avoided.
ii. CD measurements on adsorbed polyaminoacids should also be possible with modern CD spectrophotometers when sampling techniques are used. Besides corrections for adsorption flattening also corrections for scattering distortions should be made when a quantitative interpretation is wanted (*Urry and Yi, 1968; Urry et al., 1970*).
iii. In addition alternative spectroscopic techniques may be considered to obtain information about the conformation and interaction of PL with silica, such as IR, RAMAN and NMR spectroscopy.

5.4 SUMMARY AND CONCLUSIONS

In this chapter the adsorption of polycations on the surfaces of negatively charged polystyrene and silica particles was investigated as a special case of the formation of polycation-polyanion complex formation. This was done analytically (see also chapter 4) and by titration of the colloidal particles in the H^+ form with PL.HBr in which the conductivity and pH were simultaneously followed. Also proton titrations of the silica-PL system were performed.

Just as is the case with the complex formation between linear polyelectrolytes, the conductometric titration curves κ vs c_{PL} show a break. The break is also the point after which the pH remains constant. It was concluded that at the breakpoint all $-OSO_3^-$ groups of the latex and the maximal amount of $Si-O^-$ groups of the silica were bound to an $-NH_3^+$ charge of PL. The composition of the complexes in the conductometric breakpoint is not charge-stoichiometric and in the case of latex clearly superequivalent with respect to the PL charges. In the case of silica the charge composition in the breakpoint is dependent on the equilibrium pH and ionic strength. At pH 10.2 the charge (non) stoichiometry is the same as that at low pH in the PL-latex system, however the amount adsorbed is higher due to the reduced chain charge density on the PL molecules at this pH.

The main cause for the formation of superequivalent complexes is the rigidity of the colloidal particles and the relative large average distances between the charged surface groups. This is in contrast with what is generally observed for the complex formation between dissolved linear polycations and polyanions. In the latter case 1:1 complexes can be formed because both polyelectrolytes are flexible. However for dissolved systems also deviations from 1:1 stoichiometry are observed at high ionic strength.

A clear difference between the PL charged particle systems and most homogeneous linear flexible polyelectrolyte complexes is that the composition of the complex still changes (i.e. increasing adsorbed amount PL) after the conductometric breakpoint. This shows that also other than coulombic interactions, such as ion-dipole, H-bonding and hydrophobic interactions become effective in that case. This was also concluded in chapter 4 from the salt dependence of the amount of PL adsorbed. The results of the conductometric titrations (κ vs c_{PL}) are not conflicting with the mosaik charge model of *Kasper (1971)*. Comparison of flocculation results and the conductometric titration results of the PL-silica system suggest that 1:1 complexes may be formed, in contradiction with other observations. More information is needed to elucidate this.

The fraction of $-NH_3^+$, remaining free after adsorption of PL on PS-particles was estimated by conductometric titrations with polyvinylsulphate. The values found around the breakpoint adsorption: (0.3; 0.4) show again that the PL molecules adsorb in a rather flat but not entirely flat configuration.

Proton titrations of silica are strongly influenced by the presence of PL. The silanol groups become more acidic (i.e. they assume a lower apparent pK) in the presence of PL. The PL molecules adsorbed act as an effective screener of the $Si-O^-$ charges, thus promoting the dissociation of the surface groups. This effect is also observed in homogeneous polyelectrolyte complexes. The influence of the ionic strength on the charge-pH curves of silica is reversed in the presence of PL.HBr, because of the competition for surface sites between Na^+ and NH_3^+ lysine charges.

In section 5.3.7 the difficulties met with the measurement of UV and CD spectra of on silica adsorbed polyhistidine are described. Spectra of adsorbed PHis are reported.

5.5 REFERENCES

Abendroth, R.P. (1970). J. Colloid Interface Sci. *34*, 591-596.

Beychok, S. (1967), in 'Poly-α-aminoacids', Fasman, G.D. ed., M. Dekker New York.

Bleier, A. and Goddard, E.D. (1980). Colloids and Surfaces *1*, 407-423.

Bonekamp, B.C.; Schee, H.A. van der and Lyklema, J. (1983). Croat. chem. Acta *56*, 695-704.

Ciferri, A.; Puett, D.; Rajagh, L. and Hermans, J. (Jr.) (1968). Biopolymers *6*, 1019-1036.

Domard, A. and Rinaudo, M. (1980). Macromolecules *13*, 898-904.

Domard, A. and Rinaudo, M. (1981). Macromolecules *14*, 620-625.

Duysens, L.N.M. (1956). Biochim. Biophys. Acta *19*, 1-12.

Daniels, S.T. (1980) in 'Adsorption of microorganism to surfaces'. Britton, G. and Marshall, K.C. eds. J. Wiley and Sons. Chapter 2.

Eggert, A.R. (1976). Ph.D. thesis Lawrence University, Appleton Wisconsin.

Eisenlower (1982). Discussion remarks, Williams et al. (1982) in 'The effect of polymers on dispersion properties'. Tadros, Th.F. Acad. Press London, p. 373.

Gelman, R.A.; Glaser, D.N. and Blackwell, J. (1973). Biopolymers *12*, 1223-1232.

Gregory, J. (1973). J. Colloid Interface Sci. *42*, 443-456.

Horn, D. (1978). Progr. Colloid Polym. Sci. *65*, 251-264.

Ho, C.H. and Howard, G.J. (1982) in 'The effect of polymers on dispersion properties', Tadros, Th.F. ed., Acad. Press London. p. 343-359.

Juriaanse, A.C.; Arends, J. and Ten bosch, J.J. (1980). J. Colloid Interface Sci. *76*, 212-219.

James, R.O. and Parks, G.A. (1982) in 'Surface and Colloid Science' *12*, Matijević, ed., Plenum Press New York-London.

Kasper, R.D. (1971). Ph.D. thesis California Institure of Technology, Pasadena, California.

Lindquist, M.G. (1975). Ph.D. thesis Lawrence University Appleton Wisconsin.

Lindquist, M.G. and Stratton, R.A. (1976). J. Colloid Interface Sci. *55*, 45-59.

Manning, G.S. (1972). Ann. Rev. Phys. Chem. *23*, 117-140.

Manning, G.S. (1978). Q. Rev. Biophys. *11*, 179-246.

Marra, J.; Schee, H.A. van der; Fleer, G.J. and Lyklema, J. (1983), in 'Adsorption from Solution', Ottewill, R.H.; Rochester, C.H. and Smith, A.L., eds., Acad. Press, p. 245.

Michaels, A.S.; Mir, L. and Schneider, N.S. (1965). J. Phys. Chem. *69*, 1447-1455.

Norde, W. (1976). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Norde, W. and Lyklema, J. (1978). J. Colloid Interface Sci. *66*, 295-301.

Philipp, B.; Dawydoff, W. and Linow, K.J. (1982). Z. Chemie *22*, 1-13.

Polderman, A. (1975). Biopolymers *14*, 2181-2195.

Record, M.T.; Anderson, C.F. and Lohman, T.M. (1978). Q. Rev. Biophys. *11*, 103-178.

Rice, S.A. and Nagasawa, M. (1961). 'Polyelectrolyte solutions', Acad. Press London-New York.

Reinert, K.E.W. (1981). Bioel. Bioeng. (J. Elect. Anal. Chem.), *8*, 301-308.

Roe, R-J. (1974). J. Phys. Chem. *60*, 4192-4207.

Ross, P.D. and Shapiro, J.T. (1974). Biopolymers *13*, 415-416.

Schee, H.A. van der (1984). Doctoral thesis Agricultural University Wageningen, The Netherlands.

Sonntag, H. and Pilgrimm, H. (1976). Progr. Colloid and Polym. Sci. *61*, 87-92.

Sonntag, H. and Kolesnikova, R.S. (1980). Z. Phys. Chem. *261*, 226-232.

Schellman, J.A. (1975). Biopolymers *14*, 999-1018.

Scheutjens, J.M.H.M. and Fleer, G.J. (1979). J. Phys. Chem. *83*, 1619-1635.

Scheutjens, J.M.H.M. and Fleer, G.J. (1980). J. Phys. Chem. *84*, 178-190.

Tanford, C. (1965). 'Physical Chemistry of Macromolecules', J. Wiley and Sons, New York-London-Sydney.

Terayama, H. (1952). J. Polym. Sci. *8*, 243-253.

Tsuchida, E.; Osada, Y. and Abe, K. (1974). Makromol. Chem. *175*, 583-592.

Tsuchida, E.; Osada, Y. and Ohno, H. (1980). J. Macromol. Sci.-Phys., B *17*, 683-714.

Urry, D.W. and Yi, T.H. (1968). Arch. Bioch. Biophys. *128*, 802-807.

Urry, D.W.; Hinners, T.A. and Masotti, L. (1970). Arch. Bioch. Biophys. *137*, 214-221.

Yates, D.E. and Healy, T.W. (1976). J. Colloid Interface Sci. *55*, 9-19.

6 CONFORMATION OF FREE AND ADSORBED POLYLYSINE

6.1 INTRODUCTION

In the previous chapters the adsorption of PL as a highly charged polypeptide was considered. Now I will focus on the adsorption properties of PL-L and PL-DL as a function of the chain charge density, i.e. as a function of the pH above pH 7. In this pH region not only the adsorption properties change, but also the properties in solution. Above pH $\gtrsim$ 8 there appears a secondary structure in PL-L, but not in PL-DL at sufficiently low charge density and at still higher pH (above pH 11) precipitation of both stereoregular forms of PL sets in.

Except the adsorption properties at high pH, also some precipitation characteristics of both PL-L and PL-DL were studied because at very low chain charge density multilayer adsorption, i.e. precipitation on an adsorbed PL layer can take place.

The main questions to be answered in this chapter are:
i. Does the adsorption and/or precipitation behaviour of PL-L and PL-DL differ?
ii. Is there a coil to helix transition in adsorbed PL-L and what are the factors that govern conformational transitions in the adsorbed state, if any.

The precipitation behaviour was studied by means of turbidity measurements as a function of temperature, pH and time. In addition adsorption isotherms of PL-L and PL-DL at high pH and adsorbed amounts as a function of pH were determined to compare the behaviour of both stereoregular PL forms. The occurrence of conformational transitions was followed by means of proton titrations of adsorbed PL-L and PL-DL. This method is one of the few capable to give structural information of adsorbed PL in the very turbid concentrated latices.

The behaviour of polyaminoacids exhibiting secondary structure in solution in an anisotropic environment, is not only interesting from a physical-chemical point of view, but also in biology. The secondary structure of biopolymers, adsorbed at an interface may be of importance in immunological and cell recognition processes. For techniques such as immunoadsorption and the use of immobilized enzymes persistence of the native conformation in the bound state is essential.

6.2 INTERACTIONS DERTERMINING THE CONFORMATION OF CHARGED POLYAMINOACIDS

Synthetic polypeptides have been found to exist in many ordered conformations characteristic of those found in proteins (*Fasman, 1967*). In this connection, poly-L-lysine is an especially interesting homopolyaminoacid, in that it can form three conformations. At low pH, i.e. when the ε-aminogroups are protonated, the molecule is 'randomly' coiled and typical of soluble synthetic polyelectrolytes. However some local ordered conformation is probably present at low ionic strength under these circumstances (*Painter and Koenig, 1976*). See also section 3.1.2. At high pH and room temperature the ε-ammonium groups dissociate and the nearly uncharged molecule forms an α-helix. When these solutions of high pH are heated, almost immediately a precipitate forms, which has the β conformation (*Fasman, 1967; Doty and Gratzer, 1961*).

Many factors determine the conformation of a polypeptide in solution. Generally stated, the conformation of a polyelectrolyte in a dilute solution depends on intra- and intermolecular solute-solute, solvent-solute and solvent-solvent interactions. The presence of a second solute, for instance low molecular mass electrolyte, can also exert its influence. In polyelectrolyte solutions the coulombic interaction between charged segments is a major factor. This interaction is influenced by the presence of salt.

The possibility of hydrogen bond formation between the peptide carboxyl oxygen of one residue and the amide hydrogen of another can be another important factor in determining the conformation of polyaminoacids. Still other factors that play a part are: v.d. Waals, dipole-dipole, hydrophobic and steric interactions. Especially the two last mentioned are of importance because they are strongly dependent on the nature of the amino acid side chain. In the case of adsorbed polyelectrolytes, the nature of the interface and the interaction with it is important too. The ultimate conformation will be the coumpounded result. Their relative influence can be changed by varying the solvent properties and temperature. In the next sections the most important interaction forces are discussed in some detail with particular reference to the conformational properties of polyaminoacids.

6.2.1 'Non bonded' interactions

The number of possible configurations of a polypeptide chain backbone are strongly limited by constraints in the allowed angles ϕ and ψ between the N-C^{α} and C^{α}-C' bonds respectively. Short range repulsive interactions and dipole-dipole interactions between adjacent amide groups are important in this respect. The sterically allowed configurations of a polypeptide chain are also dependent on the nature of the aminoacid side chain group substituted at the C^{α} atom.

Calculated steric diagrams (Ramachandran plots) for residues with large side chains but not branched at the β carbon are essentially the same as that of alanine (CH_3 side chain) (see for example *Cantor and Schimmel, 1980*). Of course also excluded volume effects, which are dependent on the solute-solvent interactions limit the number of possible configurations of a polypeptide chain, except at θ conditions.

6.2.2 Hydrogen bond formation

A hydrogen bond is generally said to exist between a donor molecule D-H and an acceptor A, when there is evidence that the two molecules associate in a fashion specifically involving the hydrogen atom of the donor. The hydrogen bonds of most importance for protein and polypeptide structure (i.e. those in a α helix, β sheet) are those between the backbone amide nitrogens and carboxyl oxygens. Since intramolecular hydrogen bonding competes with hydrogen bonding to water molecules, the overall contribution of hydrogen bonding to the structure of polypeptides is difficult to assess.

Klotz and Franzen (1962) studied the influence of the solvent on H-bond formation choosing the solvent-dependent aggregation of N-methylacetamide as a peptide analog. In water the formation of a H-bond between two N-methylacetamide molecules appears to be thermodynamically unfavourable (ΔG° = + 13 kJ/mol) and the enthalpy of formation is nearly zero. However in CCl_4 both ΔG° and ΔH° are negative: -3.86 $kJ.mol^{-1}$ and -17.6 $kJ.mol^{-1}$ respectively. The unitary contribution (i.e. without the cratic contribution to the entropy) to the free energy of H-bond formation (ΔG°_{u}) in N-methylacetamide dimers in water amounts to +2.93 $kJ.mol^{-1}$ (see *Olander and Holtzer, 1968*). This value can be compared with the value of the same quantity obtained earlier by *Kauzmann (1959)* from thermodynamic data of *Schellmann (1955)* on the non-

ideality behaviour of urea solutions in water. The value of $\Delta G^{\circ\prime}_u$ obtained is -1.7 ± 0.4 kJ.mol^{-1} hydrogen bonds. Obviously the results of *Klotz and Franzen* and those of *Schellman-Kauzmann* are conflicting. The data of Klotz and Franzen are in my opinion more reliable because they are based on a direct spectroscopic estimation of the amount of amide protons in H-bonds, while the interpretation of Kauzmann and Schellman is deduced from non ideality effects in urea solutions. Besides this, ureum is a poorer peptide analog than N-methylacetamide. *Olander and Holtzer (1968)* discuss the data of Klotz and Franzen and Kauzmann-Schellmann in relation to the helix stability of PGA. When the non-electrostatic stabilization free energy of the α helix in PL or PGA, as obtained from potentiometric data (usually of the order of -(0.2-0.7 kJ.mol^{-1})) is compared with ΔG°_u for the formation of H-bonds in N-methylacetamide it is clear that when the peptide units in an α helix are not shielded from water, H-bonds are not a stabilizing interaction for the α helix formation, but to speak with *Olander and Holtzer (1968)*: "what, in fact, the data of Klotz and Franzen suggest is that interpeptide hydrogen bonds are so much weaker than water-peptide hydrogen bonds that they represent an overwhelming destabilizing influence."

Klotz and Franzen suggest, that when peptide groups are effectively shielded from water due to a specific high concentration of hydrocarbon-like residues, H-bonds can however contribute to the stabilization of secondary structures. The same was quoted by *Fasman (1967)* in relation to the helix stability of polyaminoacids but questioned by *Olander and Holtzer (1968)*, who argue that the effect cannot play a large role in the PGA helix.

Another point is that the uncertainty in the values of ΔG°_u for H-bonds obtained from model compounds is of the same magnitude as the entire stabilization free energy of the helix, so prediction of even the sign of the helix stabilization free energy from the sum of the individual (relative large) contributions, some stabilizing, some destabilizing, is very uncertain (*Olander and Holtzer, 1968*).

In poly-L-lysine and other water-soluble homopolyaminoacids H-bonding in aqueous solutions seems to be not unimportant in determing the stability of ordered compact conformations in water. This is because the helical PL conformation behaves indeed like a H-bonded structure in that it appears to be more stable at low temperatures than at high temperatures (*Davidson and Fasman, 1967; Hermans, 1966*). Also the greater stability of the α helix in 50% methanol supports

this. The peptide units in a PL helix should therefore be shielded from water at least partially. This can be realized because of the presence of the more hydrophobic parts of the side groups $(-(CH_2)_4)$ in PL, causing a local low dielectric constant environment.
On the contrary the polylysine β structure is more stable at higher temperatures (322 K) suggesting that hydrophobic interactions are more important in this case (*Davidson and Fasman, 1967*). In the presence of an interacting interface intra molecular hydrogen bonding can be enhanced because of the possible low local dielectric constant at the interface. For surfaces capable of forming H-bonds themselves this can be of importance for determining the structure of adsorbed polyaminoacids.

6.2.3 Hydrophobic bonding

The term hydrophobic bonding is used to describe the tendency of non polar groups to aggregate in an aqueous environment. This aggregation originates from the fact that water-water contacts are thermodynamically much more favourable than contacts between two non-polar groups or between a non-polar group and water. So, due to the aggregation of the non polar groups the extent of interaction with the surrounding water is diminished. The van der Waals attraction of non-polar groups for each other plays only a minor part in the hydrophobic effect. The hydrophobic effect arises primarily from the influence of the non-polar groups on the structure of the adjacent water (*Tanford, 1973*). Consequently, the effect is correlated with the structure of water. Therefore a quantitative description of the effect requires the choice of a water model (*Franks, 1973*). When hydrophobic bonding occurs, hydrogen bonds between water molecules are disrupted. The effect is characterized, at least at 298 K, by a relatively small, usually positive enthalpy change and a large positive entropy change in the range of 6-50 $J.K^{-1}$ per bond (*Némethy and Scheraga, 1962*). The free energy of the formation of hydrophobic bonds at moderate temperatures becomes more negative when the temperature is raised.

The importance of hydrophobic bonding for the structure of proteins and polypeptides was first pointed out by *Kauzman (1959)*. Hydrophobic bonding between the apolar part of lysine side chain residues $(-(CH_2)_4-)$ may also be a factor in determining the stability of the ordered conformations of PL-L (*Hesselink, 1973; Hermans, 1966*). However *Olander and Holtzer (1968)* state that: 'advocates of hydrophobic stabilization

(through interaction of side-chain methylenes), if they wish to be convincing, will have to reckon with the annoying fact that a relatively small fraction of the hydrophobic surface is effectively removed from contact with the solvent when the coil-to-helix transition occurs'. When this is true the stability of the helix cannot be explained in terms of hydrophobic bonding. Also *Hermans (1966)* deduced from the near equality of ΔG° and ΔH° (the non electrical part of the free energy and free enthalpy of helix formation) for PGA and PL and the normal values of pK_o for the carboxyl and amino groups that neither hydrophobic bonding nor hydrogen bonding by the *side chains* plays an important role in the stability of the α-helix. Hermans then stated that this argument does not rule out a contribution of the β and α CH_2 groups, which are present in the side chains of both glutamic acid and lysine, to the free energy of the formation of the α helix. The estimation of the contribution of hydrophobic bonds to the stabilization free energy of the polylysine α helix is further complicated by the fact that hydrophobic interactions are possible in both helix and coil forms (*Némethy and Scheraga, 1962; Hesselink, 1973*).

In the antiparallel β sheet of polylysine the lysine side chains are much more closely packed. Hydrophobic bonding is more important in this structure (*Némethy and Scheraga, 1962*) as was also confirmed experimentally for polylysine by *Pederson et al. (1971)*.

In the case of adsorption of polylysine on a hydrophobic surface as for example polystyrene, hydrophobic interactions between polylysine and the surface are of importance in determining the adsorption properties of PL as was pointed out already in Chapter 4 and 5. Also the conformational properties of PL will be influenced by the presence of an interacting surface as will be shown later. Hydrophobic bonding between PL side chains and the hydrophobic PS surface can play a role in this.

6.2.4 Ionic interactions

It is well known that the ordered structure, i.e. α-helix in uncharged polyaminoacids with ionizable side groups is disrupted (helix-coil transition) when the ionization of these side chains becomes too high. The cause of this transition is that at a certain degree of ionization the line charge density in the helix conformation exceeds that in the coil conformation. The pH and ionic strength dependent transition shows clearly the importance of ionic intractions in these polypeptides.

Charge-charge interactions are of a long range nature even when they

are shielded by the presence of low molecular mass electrolyte, as is the case in aqueous electrolyte solutions.

A theory for the charge induced helix-coil transition requires the computation of the electrostatic free energy of the helix and coil states which is a difficult matter.
Because of the mathematical difficulties involved, a detailed theory, in which the electrostatic energy of all combinations of charged and uncharged helix and coil states would have to be computed is not developed yet. Progress in this field has been made by considering both the helix and coil state as a uniformly charged cilinder, with its counterions. Since the local charge density is high, the Debije-Hückel approximation may not be applied and numerical techniques for the solution of the Poisson-Boltzmann equation are required. These computations can provide the pH-values and/or salt concentrations at which the free energy of the perfect helix is equal to that of the coil *(Poland and Scheraga, 1970)*. This is the point where the helix-coil transition will take place. Local charge effects (i.e. site binding) and specific ion adsorption, which can be of importance in practical systems have hitherto been neglected in the above approximation.

Early literature concerning the Poisson-Boltzmann description of the ionic interactions in a charged macromolecule can be found in the book 'Polyelectrolytes' by *Rice and Nagasawa (1961)*. Later calculations of the potential of a polyion in solution have been made for example by *Katchalsky (1971)*, *Fixman (1979)* and *Stigter (1975)*.

Another complicated problem is the effect of the polyelectrolyte charge on the conformation of the macromolecule, i.e. the effect on the chain flexibility and chain dimensions. A review of this matter has been given by *Nagasawa and Takahashi (1972)*.

Besides the Poisson-Boltzmann description of a polyelectrolyte, the concept of counterion condensation for the description of the interaction of small ions with linear polyelectrolytes as introduced by *Oosawa* (see *Oosawa, 1957*) can be used. This concept was developed further theoretically by *Manning (1969, 1972, 1978)*.
Counterion condensation is supposed to be governed solely by the dimension less parameter ξ, defined as:

$$\xi = \frac{e^2}{4\pi\varepsilon kTb} \qquad (6.1)$$

where e is the elementary charge, ε is the solvent dielectric constant, k is the Boltzmann constant, T is the absolute temperature and b is the average spacing between the charges on a linear polyelectrolyte molecule.

$e^2/4\pi\varepsilon kT$ is the Bjerrum length and it has the value of 0.714 nm in bulk water at 298 K. Manning showed that when $\xi < 1$ no counterions condense on the polymer, but when $\xi > 1$ counterion condensation occurs. The fraction of the line charge that is compensated by condensed counterions is $\vartheta_M = 1-\xi^{-1}$. After condensation the polyelectrolytes with the condensed envelope of counterions are still highly charged. An effective charge of ξ^{-1} remains, i.e. one charge per Bjerrum length. These charges are now screened by the counterion atmosphere. This screening can be treated then in a Debye-Hückel approximation using the effective charge density after condensation.
Fixman (1979) pointed out that from a correct application of the Poisson-Boltzmann equation to polyelectrolytes a certain version of the condensation model may be derived. The effective chain charge density determines the adsorbed amount of polylysine as will be shown later. In the calculated curves of adsorbed amount as a function of the titration charge as presented in chapter 7 counterion condensation will be included.

Besides purely coulombic interactions also specific ionic interactions can play a role in polyelectrolyte systems (see for example *Rice and Nagasawa*, Chapter 8 (1961); *Morawetz*, Chapter 7 (1965). For example, bromide binding on the peptide group of polylysine can reduce the effective chain charge density (*Ciferri et al., 1968*) and hence influence the occurrence of the helix-coil transition.

During and after adsorption of charged polyaminoacids at charged interfaces, the polyaminoacid molecules are in the overlapping electric field of the interface. This can influence the adsorption properties as well as the conformational properties of the adsorbing and adsorbed polyaminoacid.

6.3 SECONDARY STRUCTURE AND HELIX-COIL TRANSITIONS IN CHARGED POLYAMINOACIDS

Changes in the secondary structure of a polypeptide in solution can be induced by either a change in temperature or a change of the solvent. In the case of chargeable weak acid or basic polyaminoacids a change in pH can also cause a conformational change. In many cases, only small alterations in an external variable are required to bring about very dramatic secondary structural rearrangements. The pH dependent conformational transitions are also strongly cooperative. Also the introduction of an interface can disturb the delicate balance between the

various interaction forces that are responsible for an ordered conformation in solution, and hence adsorption can cause a conformational transition.

A good introduction to the theoretical and experimental aspects of helix-coil transitions in uncharged polyaminoacids has been given by *Cantor and Schimmel (1980)*. Much more elaborate treatments on helix-coil transitions in biopolymers can be found in the books of *Birshtein* and *Ptitsyn (1966)* and *Poland and Scheraga (1970)*.

6.3.1 Theories for the helix-coil transition in charged polyaminoacids

Despite the importance of charge-induced conformational transitions in biological systems, only very few theoretical studies are devoted to the effect of charge on the ordered conformations of biological macromolecules, and as far as I know no treatment at all is available for adsorbed polypeptides. This is mainly because of the great computational difficulties encountered because of the long range nature of the charge-charge interactions. Therefore temperature-induced helix-coil transitions, which are biologically clearly less important are treated theoretically extensively in literature (*Poland and Scheraga, 1970*).

Zimm and Bragg (1959) were the first who developed a statistical thermodynamical theory for the cooperative helix-coil transition in single uncharged polypeptide chains in solution. This theory defines two parameters, s and σ. Here s is the equilibrium constant for adding an amide residue to a helical segment:

$$s = \exp(-\Delta G_{conf.}/kT) \qquad (6.2)$$

σ characterizes the initiation of a helical segment, which in turn determines the sharpness of the transition:

$$\sigma = \exp(-\Delta G_{init}/kT) \qquad (6.3)$$

In the presence of an interacting interface the transition can be described by effective values of the above parameters s_{eff} and σ_{eff} (*Zhulina et al. 1980*). These effective values are then determined by the interactions with the interface. In most theories of helix-coil transitions, the interaction between dissolved polypeptides is neglected. This is worse for adsorbed polypeptides because of the high

volume fraction in the adsorbed layer.

Zimm and Rice (1960) extended the Zimm-Bragg theory for electrostatic interactions using the Debije-Hückel theory for the calculation of the electrostatic free energy. While the assumption, made by Zimm and Rice, that the total electrostatic free energy can be made up of Debije-Hückel pair interactions is probably quite poor, this theory illustrates some features and difficulties of treating the helix-coil transition in charged macromolecules (see also *Rice and Nagasawa, 1961*). In their treatment Zimm and Rice considered interactions among four units in the chain, correlating the i; (i+1); (i+2) and (i+3) units, using the nearest-neighbour Ising model to assign the statistical weights of non-ionic origin and the weight obtained from the Debije-Hückel pair interactions for the ionic contributions. Later several authors simplified the Zimm-Rice theory, mainly for practical reasons.

The s value for the uncharged coil to helix transition can be determined from the potentiometric proton titration curve of a polyaminoacid:

$$-\frac{\Delta G_{conf.}}{kT} = \ln s = 2.3 \int (pK_{app} - pK_c)\, d\alpha \qquad (6.4)$$

where pK_{app} is the measured apparent pK value and pK_c the extrapolated apparent pK for the pure coil.

A recent theory for the description of the charge-induced helix-coil transition is given by *Nakagaki and Ebert (1982)*. This theory is based on the helix-coil transition theory of *Applequist (1963)*, which is essentially a generalization of the Zimm-Bragg theory mentioned earlier. The electrostatic contribution to s is accounted for by using the Gouy-Chapman equation for plane geometry, relating the surface charge σ_o to the surface potential ψ on the helix surface.

The theories mentioned above are only of very limited value for the description of conformational transitions in the adsorbed state, especially for charge-induced transitions. Only for uncharged polypeptides there are some theoretical treatments of helix-coil transitions in the presence of an interacting interface (*Dimarzio and Bishop, 1974; Birshtein et al., 1979* and *Zhulina et al., 1980*). These authors give a treatment of the adsorption of infinitely long single polypeptide chains, thus neglecting the interaction between adsorbed chains. Therefore the results of these theories are probably of limited value for practical situations where these interactions play certainly a role as mentioned before.

In principle it should be possible to extend the existing theories for the adsorption of uncharged polymers of *Roe (1974)* and *Scheutjens-Fleer*, which take into account interpolymer interactions, for polymers exhibiting secondary structure. But then first the description of chain stiffness and adsorption of copolymers of variable composition must be incorporated in these theories. The polyelectrolyte adsorption theory of *van der Schee (1984)* is an extension of the Roe and Scheutjens-Fleer theory. The extension to the adsorption of charged chains with secondary structure and the description of charge-induced conformational transitions seems therefore attainable when the theory for uncharged polypeptides is completed.

6.3.2 Conformational aspects of poly-DL-aminoacids

In this study experiments were also performed with atactic poly-DL-lysine in order to compare the results with isotactic poly-L-lysine. Some aspects of the conformation of highly charged PL-L and PL-DL are already discussed in section 3.1. In this section I will focus on the conformational properties of (nearly) uncharged PL-DL. Racemic atactic PL-DL does not show a helix-coil transition in the region where the transition in PL-L is observed. The reason for this may be unfavourable side chain interactions in the PL-DL molecule. However, at very low charge density PL-DL may be partially helical. *Olander and Holtzer (1968)* concluded from ultraviolet extinction coefficient measurements that nearly uncharged poly-DL-glutamic acid is partially helical. Also *Gratzer (1967)* came to this conclusion on the basis of the hypochromicity observed in poly-DL-glutamic acid by *Rosenheck and Doty (1961)* and the dielectric measurements of *Wada (1962)*. However the literature on the subject is conflicting. For example *Heitz and Spach (1971)* argue that both alternating and random co poly-DL-benzylglutamate can exist in the helical form, while *Hardy et al. (1971)* assert that alternating poly-DL-benzylglutamate forms a new, as yet uncertain structure. In a later publication *Heitz et al. (1975)* conclude that strictly alternating poly-DL-benzylglutamate i.e. without any racemization occurring, can also show a new type of helix (π_{DL}) besides the α helix.

Conformational energy calculations of *Hesselink and Scheraga (1972)* indicate that the energies of left- and right handed α helical forms of alternating poly-DL-alanine are almost identical and comparable to that of the right-handed α-helical form of poly-L-alanine. The same was found to be true for poly-DL-α-aminoheptanoicacid) as a model

for uncharged poly-DL-lysine and some other poly-DL-aminoacids. Hesselink and Scheraga concluded from this that no steric hindrance to α helix formation is present for these DL copolymers.

Stulz et al. (1983) studied the tacticity and secondary structure of atactic poly-DL-leucines with FT-IR and ^{13}C NMR cp/MAS (cross-polarization/magic angle spinning). They concluded from their data that the length of isotactic blocks in these polyaminoacids was 1.5-5 monomeric units. The samples with a DP > 50 appeared to have an α-helix content of about 50-60% independent of the tacticity.

It seems very likely from the above that also atactic polyaminoacid can show some secondary structure. This can be of relevance for the precipitation behaviour of these polyaminoacids (see section 6.4.2).

6.4 PRECIPITATION OF POLY-L-LYSINE AND POLY-DL-LYSINE AT HIGH pH VALUES

It is well known that most charged polyaminoacids precipitate from aqueous solution at low degree of ionization. The insolubility of uncharged polylysine shows that the well known Flory-Huggins interaction parameter χ must exceed the value 0.5. Charged polylysine is soluble because of the strong repulsion between the chains.

The description of the precipitation of polyaminoacids can be complicated by the possible formation of crystalline or pseudo crystalline precipitates, the occurrence of which may be connected with the extent of secondary chain structure. In this respect an interesting question is whether or not there are differences in precipitation behaviour between poly-L- and (atactic) poly-DL-lysine.

The precipitation behaviour of poly-L-lysine and also poly-L-glutamic acid has been studied before bij *Puett and Ciferri (1968)* as a function of salt concentration, pH and temperature. They used their potentiometric and optical titration results (*Ciferri et al. 1968*) to judge the position of the precipitation points. *Zimmerman and Mandelkern (1975, a, b)* studied the precipitation time, i.e. the time required to observe precipitation from aqueous solution after the adjustment of certain conditions, of poly-L-glutamic acid as a function of the pH. They showed that there are two distinctly different precipitation regions, which depend on temperature, concentration and pH. Also the degree of homogeneity of the polymer samples plays an important role. In one of these regions, the α region, a variety of physical properties demonstrate that precipitation occurs without any conformational

change, while in the so called β region a major conformational transition occurs.

Poly-L-lysine displays many properties that are similar to those of PGA-L. Both are known to form gels under appropriate conditions and both form β precipitates, though it is not known yet whether also in poly-L-lysine two distinct precipitation regions exist.

6.4.1 Experimental

The precipitation experiments were performed with the unfractionated poly-L and atactic poly-DL-lysine samples obtained from the Sigma chem. corp. and described before (section 3.1). All other chemicals used were of pro analyse quality. The water used was high quality deionized water obtained from a Millipore super Q water purification apparatus.

The precipitation measurements were performed similar to the procedure of *Zimmerman and Mandelkern (1975a)* and run as follows. A stock solution of PL-DL or PL-L of 1 $kg.m^{-3}$ in 0.1 M NaBr pH 6, contained in a closed titration vessel under N_2 atmosphere, was kept at the desired temperature in a water bath. The solution was kept homogeneous by means of N_2 bubbling through it. The N_2 was CO_2-free and water vapour saturated. Then about 2 M NaOH was slowly added to the PL solution until the desired pH was reached. At this point, a portion of the solution usually 5.0 cm^3 was removed and placed in a well-closed marked test tube kept at the same temperature. Addition of NaOH to the stock PL solution was continued until a higher pH was reached and a portion of the solution was again removed. This process was continued until a sample at each desired pH from the lowest to the highest was obtained. The time at which the pH of each sample was adjusted was noted and considered as time zero for each sample. At regular time intervals the $OD_{10\ mm}^{550\ nm}$ from an aliquot (0.5 cm^3) of each sample was then measured with a Beckmann 3600 spectrophotometer. In this way the $OD_{10\ mm}^{550\ nm}$ could be plotted as a function of time for each pH value. Calibration of the pH electrodes (Schott combined glass-Ag/AgCl) occurred at the measuring temperature with titrisol buffers (Merck) pH 7.00 ± 0.02; pH 10.00 ± 0.05 and pH 11.00 ± 0.05. The pH measurements were performed with an Electrofact 36060 pH/mV meter.

6.4.2 Results and Discussion

When turbid PL solutions, i.e. after phase separation, are looked at, the turbid solutions, especially at high PL concentration, appeared to have a more or less gelly character, so no flaky precipitates are observed.

The results of the precipitation measurements performed by us are shown in fig. 6.1, 6.2 and 6.3. In fig. 6.1 the measured OD is plotted against time at 303.15 K and 313.15 K for different PL samples and different pH values. In fig. 6.2 the OD after a chosen incubation time is plotted against the pH for PL-L and PL-DL samples at 303.15 and 313.15 K, and in fig. 6.3 the time after which an arbitrarely chosen turbidity was reached is plotted against the pH, for the same PL samples as in fig. 6.1 and 6.2. The precipitation time plotted in fig. 6.3 may be compared with the precipitation times observed visually by Zimmerman and Mandelkern in their experiments on PGA.

Determinations of the amount precipitated at 293 K in an analogous way as the amount PL adsorbed, will be reported in section 6.5.3.2.

Above pH 11.0 both PL-L (DP 192) and PL-L (DP 1683) showed an appreciable precipitation after 16 h. This is in line with the findings of among others *Ciferri and Puett (1968)* who found precipitation of PL-L at 298 K above pH 11.3. However in one series of measurement we did not find any visual turbidity of PL-L (DP 1683) above pH 11.0. The reason for this is not clear to me.

Precipitation measurements with a PL-L (DP 240) sample which appeared to have about 1 residual blocking group (as judged from the UV sprectrum) per PL chain showed a precipitation behaviour markedly deviating from the pure samples: The pH at which the precipitation started was about 1 pH unit lower than the precipitation pH of a corresponding PL sample without blocking groups.

Zimmerman and Mandelkern (1975a) showed that there exist quantitative differences in the precipitation behaviour of unfractioned PGA(M_w/M_n=1.1) and fractionated samples (M_w/M_n = 1.0), although also the unfractionated PGA samples show the two precipitation regions mentioned before. Thus despite the fact that our experiments with PL have been done with unfractionated samples, valuable information concerning the precipitation characteristics can be obtained from them.

In contrast with the gradual increase in turbidity with time for the PL-L samples, the curves for PL-DL show initially a rather steep increase in turbidity after which further increase is more or less

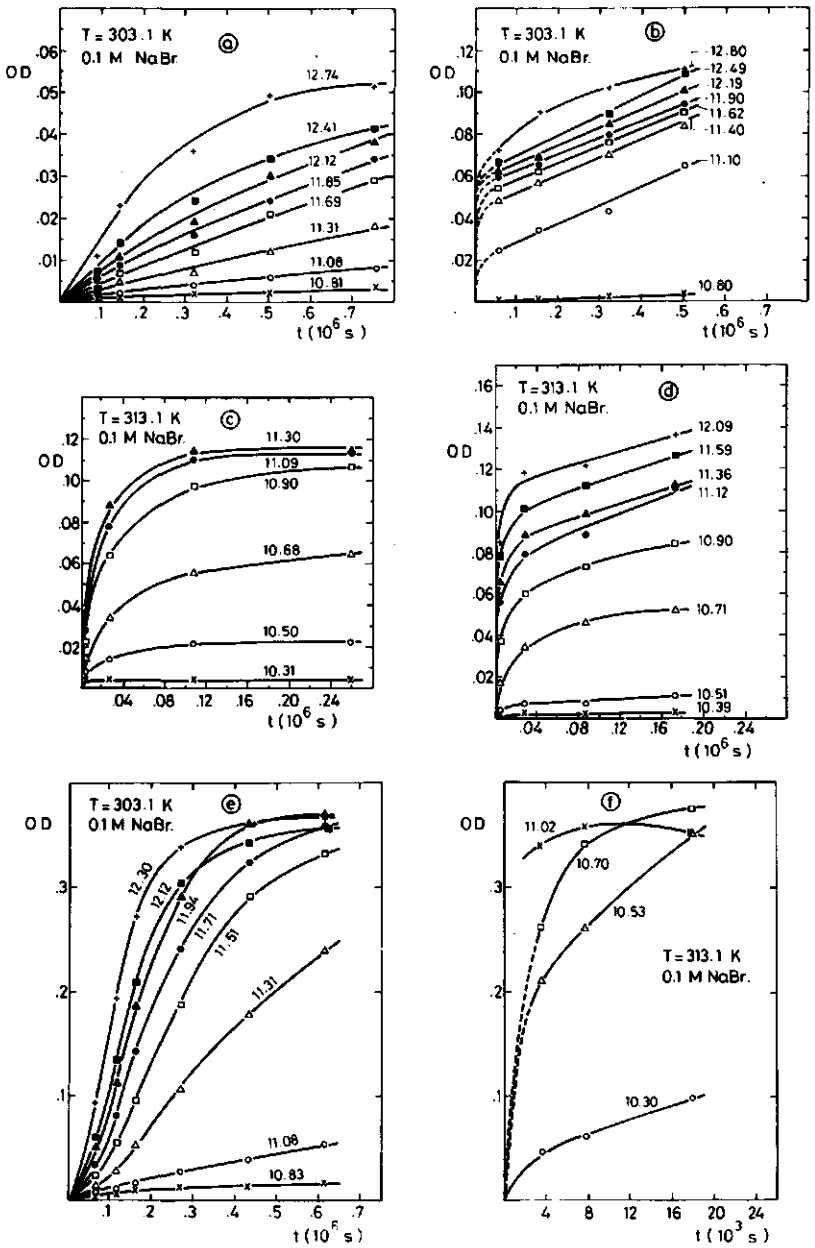


Fig. 6.1 Precipitation of polylysines as a function of time at various pH values and two temperatures. Precipitation was monitored by measurement of the absorbance at 550 nm; optical pathway: 10 mm.
c_{PL} = 1.00 kg.m^{-3}; pH values are indicated.
a. PL-L (DP 192); b. PL-DL (DP 308); c. PL-L (DP 192);
d. PL-DL (DP 308); e. PL-L (DP 1683); f. PL-L (DP 1683).

linear (compare fig. 6.1a and b). However, at 313.15 K the initial slopes of the curves is almost the same for both PL-L and PL-DL (see fig. 6.1c and d). At longer incubation times the difference between the curves of PL-L and PL-DL observed at 303.15 K is almost absent at 313.15 K.

The observed increase in turbidity with time can be caused both by growth of aggregates and the agglomeration of aggregates. Therefore an increase in turbidity does not necessarily mean an increase of the precipitated amount with time.

Measurement of the PL concentration after removal of the precipitate by centrifugation showed that an increase in turbidity corresponds to an increasing amount of precipitated PL-L (DP 192) or PL-DL (DP 308) at least at 313 K. At this temperature and pH 10.7 the fraction precipitated was about 30% and 50% for PL-DL and PL-L respectively. At high pH (above 11.5) the precipitated fraction was 80-85% for both PL-L and PL-DL. The quantitative relation between OD and precipitated amount is very likely to vary for each sample.

The precipitation curves of PL-L (DP 1683) show much higher turbidities than those of PL-L (DP 192). At 313.15 K the maximum turbidity is reached much faster than in the case of PL-L (DP 192). The precipitated amount PL-L (DP 1683) at 308.9 K appeared to be constant above pH 11.3 and amounted 95%. The observed differences between PL-L (DP 192) and PL-L (DP 1683) are in line with the expectations for increasing phase separation with increasing molecular mass.

The differences observed by us between the OD-time curves of PL-L and PL-DL point in the direction of differences in the growth kinetics of the aggregates. These differences can only originate from the difference in stereoregularity between PL-L and PL-DL.

From fig. 6.2 one can see that the pH, where the precipitation of PL starts at 303.15 K is 10.9 ± 0.1 for PL-L (DP 192) and for PL-DL (DP 308), and somewhat lower (pH = 10.6) for the high molecular mass PL-L (DP 1683) as expected for a phase separation phenomena. At 313.15 K the initiation of the precipitation starts at clearly lower pH values: pH 10.5 ± 0.1 for PL-L (DP 192) and PL-DL (DP 308) and pH 10.1 ± 0.1 for PL-L (DP 1683). This decrease in initiation pH was also found by *Puett and Ciferri (1968)*.
As these authors showed, the decrease in initiation pH is essentially an electrostatic effect caused by the decrease in pK_o with increasing temperature. In the temperature range investigated, the precipitation takes place at the same charge density and therefore at lower pH values

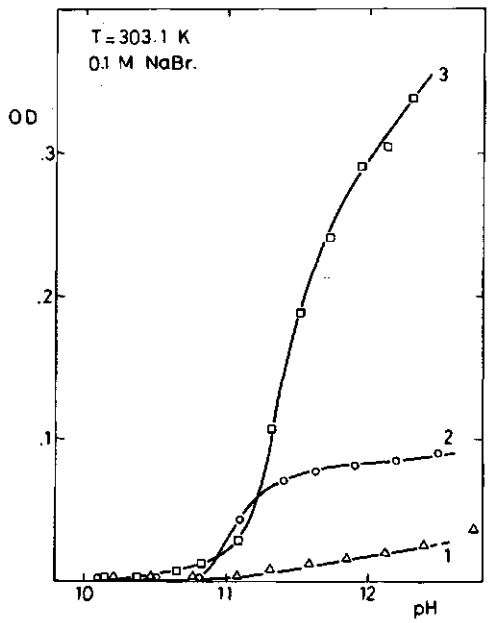


Fig. 6.2.1 Precipitation of poly-L-lysine and poly-DL-lysine as a function of pH at
T = 303.15 K.
c_{PL} = 1.0 kg.m^{-3}; 0.1 M NaBr.
1. PL-L (DP 192) t = 90 h; 2. PL-DL (DP 308) t = 90 h;
3. PL-L (DP 1683) t = 75h.

with increasing temperature. Unfortunately in our case the degree of dissociation cannot be calculated accurately enough from the available data, because the corresponding titration data are lacking.
It is remarkable that within experimental error the initiation of the precipitation occurs at the same pH value for both PL-L and PL-DL at 303.15 K as well as 313. 15 K. At 313.15 K the shape of both curves is also the same over the whole pH region. At the precipitation pH the degree of dissociation of PL-L and PL-DL can be different. This is because of a possible difference in pK_{app} of both polyelectrolytes (see section 6.6.4.2). The turbidity of PL-L (DP 1683) exceeds greatly the values found for the low molecular mass samples as stated before. Probably bigger aggregates are formed.

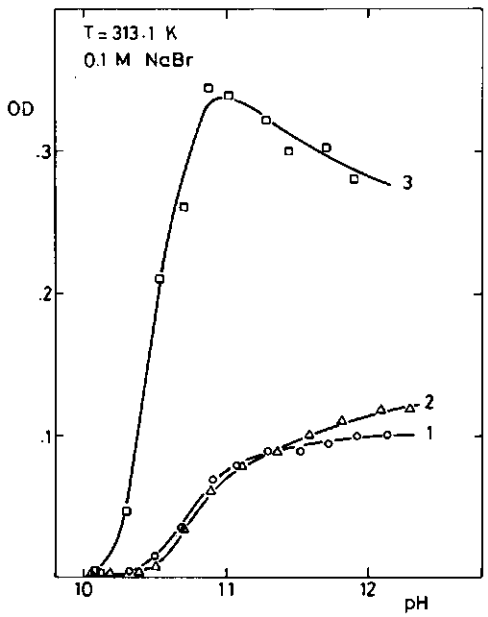


Fig. 6.2.2 Precipitation of poly-L-lysine and poly-DL-lysine as a function of pH at T = 313.15 K.
c_{PL} = 1.0 kg.m^{-3}; 0.1 M NaBr.
1. PL-L (DP 192) t = 7.5 h; 2. PL-DL (DP 308) t = 8.0 h;
3. PL-L (DP 1683) t = 1.0 h.

Another question is whether there are already soluble PL aggregates present before the observed precipitation point. This can be of importance for the adsorption properties of PL. Some indications for the existence of such soluble aggregates at pH > pK_o (T = 288 K) were found by *Propokova and Ciferri (1972)* from the electrokinetic behaviour of PL-L solutions at high pH. The results may however be an artifact of the method (*Propokova and Ciferri, 1972*).

At high pH values and T > 298 K the β-form is the thermodynamically most stable conformation of PL-L. At 278 K the α helix is the most stable comformation at high pH values (*Davidson and Fasman, 1967); Pederson et al., 1971*). If poly-L-lysine in the coil conformation is brought onto high pH with base at 322 K, the polyaminoacid assumes directly the β conformation with no detectable α helical intermediate.

Between 298 K and 322 K addition of base to 'coil' PL-L gives first an α helical PL-L polymer followed by slow conversion to the β structure (via a coil-like intermediate). Then the formation of β structure is followed by precipitation with conservation of the β structure (*Davidson and Fasman, 1967*). In contrast with the result of *Zimmerman and Mandelkern (1975a)* on PGA, the precipitation time - pH curves of poly-L-lysine and also poly-DL-lysine (fig. 6.3.1 and 6.3.2) do not show any evidence for the existence of two distinct precipitation regions. Poly-L-lysine shows only what Zimmerman and Mandelkern denote as β precipitation and no α precipitation, the latter being nucleation controlled. The above is concluded from the fact that there is no abrupt change in the pH dependence of the precipitation in the investigated pH region and also from the gradual decrease in precipitation time when the temperature is increased from 303 K to 313 K. Apparently in the case of PL-L the rate at which the random coil intermediate is generated is greater than the rate of nucleation over the whole pH region, resulting in a precipitate in which the PL-L molecules are in the β structure.

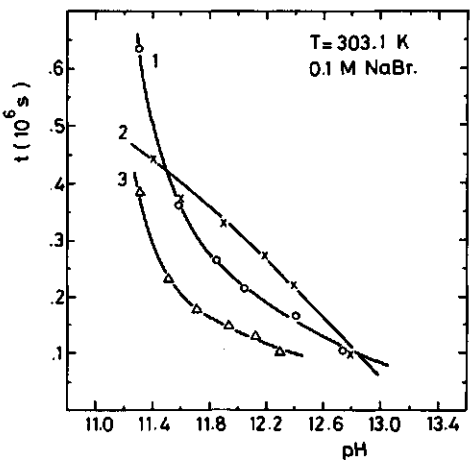


Fig. 6.3.1 Time dependence of the precipitation of poly-L-lysine and poly-DL-lysine.
c_{PL} = 1.0 kg.m^{-3}; T = 303.15 K; 0.1 M NaBr.
1. PL-L (DP 192) t($OD^{550\ nm}_{10\ mm}$ = 0.015) vs pH.
2. PL-dL (DP 308) t(" " = 0.08) vs pH.
3. PL-L (DP 1683) t(" " = 0.16) vs pH.

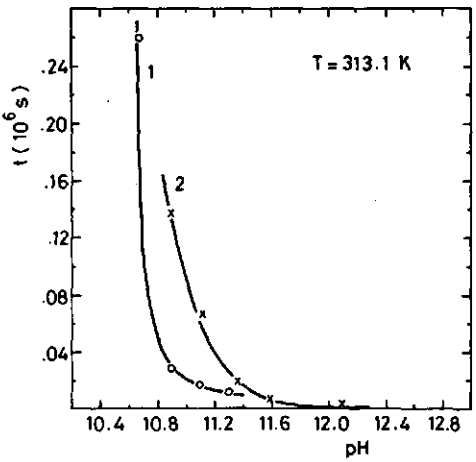


Fig. 6.3.2 As fig. 6.3.1 but at T = 313.15 K.
1. PL-L (DP 192) $t(OD_{10\ mm}^{550\ nm} = 0.065)$ vs pH
2. PL-DL (DP 308) t(OD " = 0.08) vs pH.

Both the α-β conversion and precipitation of PL-L have qualitatively the same pH and temperature dependence (above 293 K), i.e. the rates of both processes are increased when the pH or temperature is increased (see *Davidson and Fasman (1967)* for the pH and T dependence of the α-β conversion). This is not surprising because in both processes the same interactions play a role. As already concluded from the precipitation time-pH curves at 303 K and 313 K, the α-β conversion rate must be faster than the precipitation rate. A quantitative comparison of the precipitation times found here and the α-β conversion rates described in the literature is not possible at the moment, because both processes depent strongly on such experimental conditions as PL concentration molecular mass, and electrolyte concentration.

The precipitation times of PL-L and PL-DL do not differ much, also the decrease in precipitation time with increasing temperature or pH lies in the same range for PL-L and PL-DL. Although PL-DL may be partially helical at very low charge density (see 6.3.2), α-β conversion as it takes place in PL-L is not very likely to occur in PL-DL. Therefore the similarity in the precipitation behaviour of PL-L and PL-DL supports the earlier conclusion that α-β conversion via an coil intermediate is faster than the precipitation step itself in PL-L. When a

coil conformation already exists before precipitation as is probably the case with PL-DL, the initial precipitation rate can be higher as can be seen from fig. 6.1.6. However at 313.15 K this difference in initial precipitation rate between PL-L and PL-DL is practically disappeared as can be seen from fig. 6.1.c and 6.1.d. Probably this is a consequence of the fact that the PL-L α helix is unstable at this temperature.

Now the question arises whether the structures of the precipitates of PL-L and PL-DL are the same or different. Poly-DL-lysine cannot form crystalline precipitates because it is an atactic polymer. The precipitate will be amorphous probably a concentrated liquid phase in equilibrium with a very dilute one. On the contrary, poly-L-lysine is isotactic and can form a crystalline precipitate (solid-liquid phase separation) in which the polylysine molecules are in the β conformation. The fact that the precipitation pHs and temperature dependence of the precipitation are not very much apart for both polylysines, suggests that the precipitate of poly-L-lysine is also largely amorphous.

To unravel the precipitation behaviour of PL-L and PL-DL further, more direct information about the PL-L and PL-DL conformation in the precipitate and the degree of order in the precipitate is needed. This information can be obtained for example with techniques such as Laser Raman spectroscopy and X-ray diffraction. Also much more information on the precipitation process itself is needed. Especially the volume fraction dependence as a function of pH, salt concentration and temperature should be determined in order to make it possible to construct phase diagrams and to allow for a more complete interpretation. The precipitation data discussed above are nevertheless very valuable for the interpretation of adsorption experiments at low PL chain charge density.

6.5 ADSORPTION OF PARTIALLY CHARGED POLY-L-LYSINE AND POLY-DL-LYSINE ON POLYSTYRENE

6.5.1 Materials

The poly-L-lysine and atactic poly-DL-lysine samples were the same as described before. The PS latices used are described in section 3.2. All other chemicals used were of analytical grade. The water used was distilled once or conductivity water obtained from a Millipore Super Q water purification set-up.

6.5.2 *Determination of the PL adsorption on PS particles as a function of the pH in solution*

Adsorption experiments at high pH performed without the use of buffers which could influence the adsorption, are complicated by the disturbing influence of atmospheric CO_2 on the solution pH value. Therefore contact of the high pH PL/latex solutions with the air was avoided as much as possible.

The amount of base needed to reach a desired pH was first calculated on a basis of the total amount PL present and proton titration data of PL. The pH was measured (only) after equilibration (16 h) and centrifugation of the sample. Immediately after opening of the centrifuge tube a sample for the PL concentration determination was taken and instantaneously thereafter the pH was measured.

Measurements with PL-L and PL-DL were performed simultaneously. The same was done for adsorption series at different ionic strength. In this way the effect of systematical errors can be suppressed.

The preparation of samples for adsorption series at high pH was done as follows: To a 8 cm^3 polyethylene tube with screw cap known amounts of water, PL stock solution (pH 6) and NaBr solution pH 6 were added. Then a known amount of 0.1 M NaOH was added, immediately followed by the addition of 1.0 cm^3 PS latex (pH 6). The tube was then closed immediately and rotated end over end for 16 hours to attain adsorption equilibrium. The total volume of the sample was always 5.0 cm^3. The PL concentration determination was performed as described before (chapter 4) except for one difference: 50 mm^3 0.1 M HCl was added to bring the sample at a pH below 6, i.e. to obtain fully charged polylysine.

Adsorption samples with different starting concentrations of PL were prepared in a few separate series to judge the variation of the adsorbed amount with the equilibrium PL concentration. The average degree of dissociation of PL in an adsorption sample can be calculated from the added amount of base and the measured equilibrium pH.

Besides the adsorption measurements as a function of the equilibrium pH, adsorption isotherms were also determined as follows. Before mixing separate stock solutions of PL, NaBr and latex were brought to the desired high pH with NaOH. Then known amounts of these solutions were pipetted into polyethylene tubes as described above, to obtain a series of increasing PL concentration. The subsequent sample handling was the same as described above.

6.5.3 *Adsorption isotherms of poly-L-lysine and poly-DL-lysine at high pH*

In section 4.3.4 the adsorption characteristics of fully charged poly-L- and poly-DL-lysine were discussed. Now the adsorption isotherms of these polypeptides at high pH on negatively charged PS latex particles will be dealt with. In fig. 6.4 characteristic adsorption isotherms of PL.HBr-L and PL.HBr-DL adsorbed on PS at pH 10.7 ± 0.1 are plotted. For comparison an isotherm of PL.HBr-L at pH 6 is also given. All isotherms are of the high affinity type, although they are somewhat more rounded at high pH. It is not certain whether the plateau values found here are really plateaus. At higher PL volume fractions phase separation is possible resulting in multilayer adsorption.

The observed plateau adsorption of both PL-L and PL-DL is about 4 times higher than at α = o and not measurable different for PL-L and PL-DL. The somewhat higher adsorption values observed for PL-DL at higher equilibrium concentrations is caused by the accidental higher pH (within 0.1 pH unit) of the corresponding adsorption samples.

The plateau adsorption values observed are of the expected magnitude for uncharged polymers. The large increase in adsorption observed when the chain charge density is decreased is caused by:
(i) Decrease of the electric repulsion between polylysine segments in the adsorbed layer. The effect is similar to the effect of addition of electrolyte as discussed in section 4.3.6.
(ii) Decrease of the solvent quality.
The amount of adsorption at pH 10.7 is about 1.5 times the monolayer coverage while at pH 6 the adsorption is much less than monolayer coverage. Hence the fraction of segments in (short) loops and tails is much higher at high pH.

The increased roundedness of the isotherms at high pH can be due to a more pronounced polydispersity effect when the solvent quality is decreased, but also to variations of α with surface coverage as theoretical calculations of *Evers (1984)* for weak acid polyelectrolyte adsorption suggest. The fact that also at high pH PL-L and PL-DL have very similar adsorption characteristics shows that is is not justified to draw conclusions about the secondary structure of PL-L in the adsorbed layer on the basis of plateau adsorption values of PL-L only.

The fact that PL-L and PL-DL have the same adsorption characteristics at high pH shows that in this case the secondary chain structure

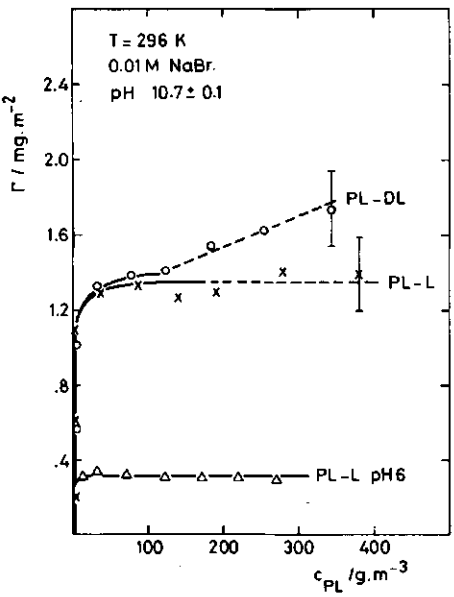


Fig. 6.4 Adsorption isotherms of poly-L-lysine.HBr (DP 240) and poly-DL-lysine.HBr (DP 240) at high pH on PS latex M_2 (σ_o = -60 mCm^{-2}) electrolyte 0.01 M NaBr. A corresponding adsorption isotherm at pH 6 is also drawn.

has no influence. Apparently the solvent interaction parameter and adsorption energy per segment are about the same for the two PL forms and these parameters are dominating the adsorption. That χ is about the same for PL-L and PL-DL follows from the similarity in precipitation behaviour. $\chi_{s,eff}$ of PL-L and PL-DL will be the same when there is a preferential adsorption of coil segments. As coil segments are on the average positively charged and the surface is negative, this is likely to be the case. It will be clear that more information is needed to clarify the structure of adsorbed PL. To this end proton titrations of adsorbed PL-L and PL-DL have been performed. They will be discussed in section 6.6.

6.5.3.1 Adsorbed amount as a function of the solution pH

In figs. 6.5, 6.6 and 6.7 the amounts of PL.HBr-L and PL.HBr-DL adsorbed as a function of the solution pH are plotted for various poly-lysine samples and salt concentrations. The adsorptions in these figures

are about plateau adsorption, i.e. they are maximum adsorptions or are between the middle of the rounded part in the isotherm and the maximum adsorption. The increase in adsorbed amount with increasing pH is clearly demonstrated. The shape of the curves is at least qualitatively as theoretically expected, as will be shown in chapter 7. The adsorption as a function of the pH is reversible, that is: after adsorption at pH 11.00 we found desorption to occur when the pH was lowered to 6 down to the adsorption value at pH 6.

As one can see from fig. 6.5 the curves for PL-L and PL-DL are not measurably different, also not in the helix-coil transition region. Again it is seen that at any α the secondary structure of PL is not important in determining the adsorbed amount.

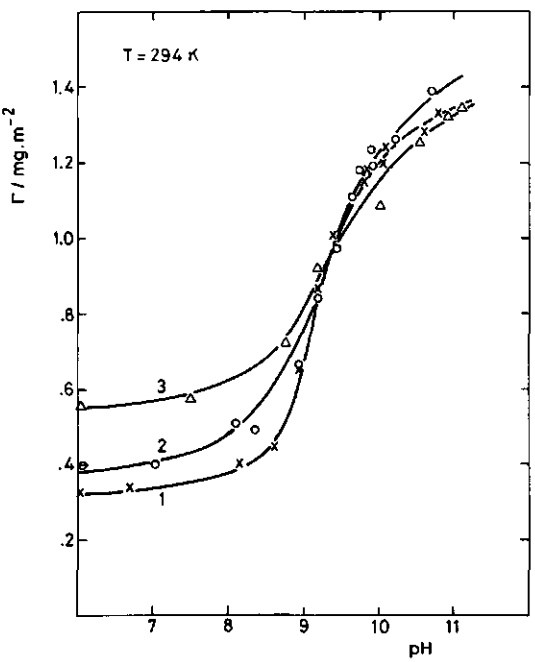


Fig. 6.5 Adsorption of poly-L-lysine.HBr (DP 192) and poly-DL-lysine.HBr (DP 240) as a function of pH on PS (latex M_4; σ_o = -42 mC.m^{-2}).
1. PL-L, 0.01 M NaBr; 2. PL-DL, 0.01 M NaBr; 3. PL-DL, 0.1 M NaBr.

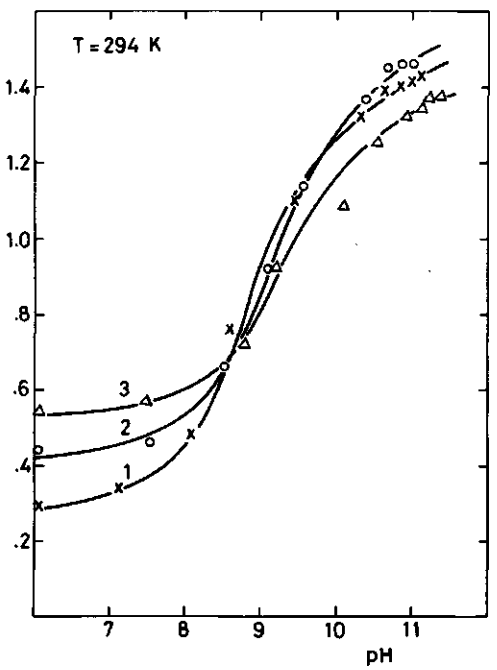


Fig. 6.6 Adsorption of poly-DL-lysine.HBr (DP 240) on PS (latex M_4; σ_o = -42 mC.m^{-2}) as a function of pH. 1. 3.10^{-3} M NaBr; 2. 1.10^{-2} M NaBr; 3. 1.10^{-1} M NaBr.

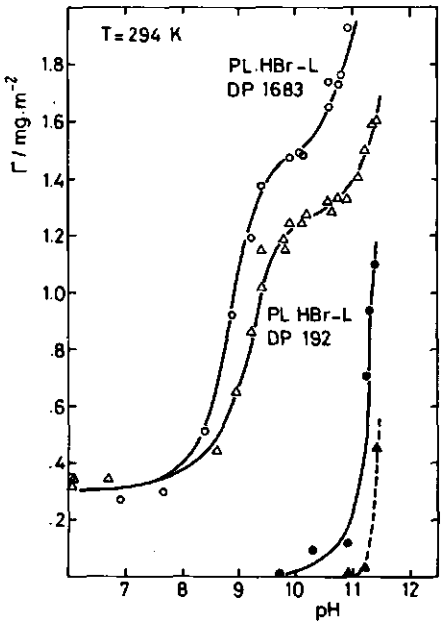


Fig. 6.7 Molecular mass dependence of the adsorption (open symbols) of PL.HBr-L as a function of the pH. 0.01 M NaBr; latex M_4 (σ_o = -42 mC.m^{-2}). Filled symbols: precipitation of PL.HBr-L as a function of the pH in the absence of adsorbent.

The effect of low molecular mass electrolyte on the adsorbed amount is clearly present at low pH but almost absent at high pH. This shows that coulombic interactions in adsorbed PL at high pH are of minor importance as expected. At still higher salt concentrations however, specific dehydration effects may become important in determining the adsorption and multilayer adsorption (i.e. precipitation) properties of PL. Although the effect of the ionic strength is small at pH 11.0, the amount adsorbed at constant pH is somewhat lower at 0.1 M NaBr than at lower ionic strength (see fig. 6.5 and 6.6). This reversed salt effect is due to the fact that at constant pH the degree of dissociation decreases with increasing ionic strength.

In fig. 6.7 the Γ-pH curves and the corresponding precipitation curves are plotted for PL-L (DP 192) and PL-L (DP 1683). Whereas at low pH (α = 0) there is no molecular mass dependence of the adsorption

above DP 192, this dependence is clearly observed at high pH. The amount adsorbed of PL-L (DP 1683) at pH 10 is significantly higher than the value for PL-L (DP 192); 1.5 $mg.m^{-2}$ and 1.2 $mg.m^{-2}$ respectively. This is a consequence of the lower solvent quality at this pH. Above pH 10.3 for PL-L (DP 1683) and pH 10.9 for PL-L (DP 192) multilayer adsorption (i.e. phase separation) occurs at about the same pH values as phase separation starts in solution (see also section 6.5.3.2). For uncharged polymers such a behaviour around the precipitation point is expected (*Silberberg, 1972*). The shape of the adsorption curves shown in fig. 6.7 is expected theoretically for the adsorption of weak polyelectrolytes from a bad solvent ($\chi > 0.55$) (*Evers, 1984*).

At 293 K and 0.01 M NaBr the pH values where the precipitation of PL-L DP 1683 and DP 192 starts are almost the same as the values found by us at 303 K and 0.1 M NaBr. This is at least qualitatively due to two compensating effects: A decrease of the precipitation pH with increasing temperature as discussed in section 6.4.2 and an increase of the precipitation pH with increasing electrolyte concentration (*Puett and Ciferri, 1968*).

6.5.3.2 Phase separation in solution and near an interface

As shown in fig. 6.7 phase separation in solution and in the presence of an interacting interface sets in at about the same pH. However, the mean degree of dissociation $\bar{\alpha}$ of PL in the presence of PS latex can differ from that in the solution at the precipitation pH, because the average pK_{app} of adsorbed PL is higher than that of PL in solution at the same overall concentration. The calculation of $\bar{\alpha}$ from the amount NaOH added, pH and total amount of PL present is possible but not accurate enough to allow for conclusions about differences in $\bar{\alpha}$ at the precipitation pH in the two cases.

Because α of PL in the outer adsorption layers is not very different from α in the bulk (as will be shown in section 6.6.4.2), no appreciable precipitation on the surface will occur when the conditions for phase separation in the bulk are not fullfilled. *Silberberg (1972)* analyzed theoretically the multilayer adsorption of uncharged polymers. He concluded that only at close to ϑ conditions ($\chi/\chi_\vartheta = 0.99$) or a PL volume fraction in the bulk, ϕ_* close to the critical one at $\chi = \chi_\vartheta$, about one additional polymer layer could be formed. Further growth occurs only at worse than ϑ conditions. However this is probably only valid for very long chains. In the case of shorter chains (DP < 100) thicker layers

can be formed at χ values close to the critical one (*van der Schee, 1984*). It will be clear that charge interactions complicate the picture in the PL-latex system. Nevertheless the results shown here are not conflicting with the predictions of Silberberg.

In the absence of precipitation the results in fig. 6.7 suggest the existence of (pseudo) plateaus values for the adsorption of PL of about 1.3 and 1.5 $mg.m^{-2}$ for DP 192 and DP 1683 respectively. As said before, the high adsorbed amount compared with pH 6 must be due to a higher fraction of segments in loops and tails. Theoretical predictions for the adsorption of weak polyelectrolytes from a good solvent, as a function of pH, show a maximum in the case of charge contrast between adsorbents and adsorbate (*Evers, 1984*). Such a maximum is not observed here, probably because precipitation occurs.

At 294 K the α-helical structure which is present in solution above pH 10 is slightly more stable than the β structure, and precipitation without conversion to the β structure might occur in the presence of already adsorbed PL. There may be different kinetic pathways operative in the case of precipitation from solution or in the presence of an adsorbing interface. In the latter case only growth of an already adsorbed layer has to be occur, in the former case also (homogeneous) nucleation can play a role.

6.6 CHARACTERIZATION OF FREE AND ADSORBED POLYLYSINE BY POTENTIOMETRIC PROTON TITRATIONS

6.6.1 Introduction

Potentiometric proton titration is an often used method for studying the charge dependent conformational transitions in mostly aqueous solutions of ionizable polyaminoacids (see also section 3.1.2). Poly-L-glutamic acid and poly-L-lysine are the most studied ones.

Studies concerning the (thermodynamic) characterization of conformational transitions in poly-L-lysine dissolved in aqueous solvents by means of proton titrations have been performed by: *Hermans, J., 1966; Puett et al., 1967; Ciferri et al., 1968; Ptitsin, 1971; Grourke and Gibbs, 1971; Pederson et al., 1971; Barskaya, 1971; Conio et al., 1974; Cosani et al., 1974* and *Tseng and Yang, 1977*. Generally, comparable proton titration data of these authors are in reasonable agreement. The intrinsic pK values found by these authors is generally lower than those reported here. This is inherent to the extrapolation procedure

to α = 1 used by these authors, i.e. the values reported in the literature are not based on determinations of pK_o values of a monomer analog as is done here. Proton titrations of atactic poly-DL-lysine have been reported by *Chou and Scheraga (1971)*.

Proton titrations of poly-L-aminoacids in interaction with solid-liquid interfaces or membranes are not reported yet in the literature. In this study the proton titration technique was used to characterize the conformation of adsorbed polylysine.

6.6.2 Principle of the method

Because of the high charge densities which can build up on a polyelectrolyte chain, the titration behaviour of a weak polyacid or polybase in aqueous solution deviates from that of the corresponding monomer analogs. The dissociation equilibrium of an isolated ammonium group in PL can be written as:

$$R - NH_3^+ \rightleftarrows R - NH_2 + H^+ \qquad (6.5)$$

The thermodynamic dissociation constant $K_{a,m}$ is then

$$K_{a,m} = \frac{(R\text{-}NH_2)(H^+)}{(R\text{-}NH_3^+)} \qquad (6.6)$$

where parentheses denote the activity of each species.

Since the activities of the species $R\text{-}NH_3^+$ and $R\text{-}NH_2$ are not known, but only their analytical concentrations (in brackets) the dissociation constant $K_{o,m}$ defined by

$$K_{o,m} = \frac{[R\text{-}NH_2](H^+)}{[R\text{-}NH_3^+]} \qquad (6.7)$$

is used instead of $K_{a,m}$, which is a true intrinsic constant. The m in the subscript denotes the medium in which the groups are. On the contrary $K_{o,m}$ is not a true intrinsic K, since it is still dependent on the ionic strength and the species concentration. In principle the thermodynamic constant $K_{a,m}$ valid for $-NH_3^+$ groups of PL in a certain chemical environment can be obtained by extrapolation of $K_{o,m}$ values to zero ionic strength and zero concentration of the component under investigation.

Equation 6.7 can be written as:

$$pH = pK_{o,m} + \log \frac{\alpha}{1-\alpha} \qquad (6.8)$$

in which α is the degree of dissociation of the ammonium groups defined by

$$\alpha = \frac{[R\text{-}NH_2]}{[R\text{-}NH_3^+]+[R\text{-}NH_2]} \qquad (6.9)$$

and $pK_{o,m} = -\log K_{o,m} = 0.434 \frac{\Delta G_m^o}{RT}$

$pK_{o,w}$ is used when water is the solvent, $pK_{o,s}$ for an aminogroup at an interface. ΔG_m^o is the standard free enthalpy change of the dissociation in the medium investigated. In this derivation activity effects other then for H^+ are ignored.

In a poly-cationic acid the dissociation of each cationic group depends also on the electric field of neighbouring other cationic groups. Generally, the free energy of dissociation now consists of an electrical term ΔG_{el} in addition to ΔG_m^o. This can be taken into account by modifying (6.8) into:

$$pK_{app} = pH - \log \frac{\alpha}{1-\alpha} = \frac{0.434}{RT} (\Delta G_m^o - \Delta G_{el}) \qquad (6.10)$$

in which pK_{app} is the apparent dissociation constant, related to the sum of the standard free enthalpy change ΔG_m^o and the additional electrostatic free enthalpy change ΔG_{el}. In (6.10) ΔG_{el} is the electrical reversible isothermal work, to bring a proton from infinity to the surface of the macroion. It follows now that

$$pH = pK_{o,m} + \log \frac{\alpha}{1-\alpha} - \frac{0.434}{RT} \Delta G_{el} \qquad (6.11)$$

Because of the definition of ΔG_{el}, this quantity is equal to the change of the electrostatic free enthalpy G_{el}, of the macroion, when the charge of the polymer is increased by one unit. Equation 6.11 is only valid when all titratable groups contained in the definition of α have the same $pK_{o,m}$. This is for biopolymers not the case and as will be shown later on also not for adsorbed homopolyelectrolytes.

When Z_H is the amount of protons dissociated from the cationic

polyelectrolyte, ΔG_{el} can be written as

$$\Delta G_{el} = - \frac{\partial G_{el}(Z_H)}{\partial Z_H} \qquad (6.12)$$

It follows now that

$$\frac{G_{el}(\alpha)}{Z_{H,max}} = - 2.303\ RT \int_{\alpha}^{1} \{pH - \log \frac{\alpha}{1-\alpha} - pK_{o,m}\}\ d\alpha \qquad (6.13)$$

where $\alpha = Z_H . Z_{H,max}^{-1}$ in which $Z_{H,max}$ is the maximum number of ionizable groups with the same $pK_{o,m}$ value.
One can in principle obtain $pK_{o,m}$ from a plot of pK_{app} vs α by extrapolation to $\alpha = 1$. The electrostatic free enthalpy G_{el} of the polyelectrolyte can be calculated from the area under the graph $(pK_{app} - pK_{o,m})$ vs α.

The total free enthalpy of an adsorbed or dissolved polyelectrolyte as a function of pH can be directly obtained from proton titration data as was shown by *Pfeil and Privalov (1976)*. Generally this free enthalpy is a function of composition, P, T, pH and ionic strength. From the equation

$$dG = (\frac{\partial G}{\partial N})_{P,T} dN = \mu_H\ (\text{polyelectrolyte}) dN = \mu_H\ (\text{solution})\ dN$$
$$= (\mu_H^o - 2.303\ RT.pH)\ dN \qquad (6.14)$$

in which N is the average charge on the polyelectrolyte chain, it follows after integration:

$$G(N) = G(N^o) - 2.303\ RT \int_{N^o}^{N} pH\ dN \qquad (6.15)$$

where $G(N^o)$ is the free enthalpy in some reference state in which the charge on the polyelectrolyte is FN^o (see *Pfeil and Privalov, 1976* and *Norde and Lyklema, 1984*).

Equation 6.10 is very suitable for analyzing the potentiometric titration data of charged polyaminoacids in solution and in the adsorbed state.

$G_{el}(\alpha)$ can also be expressed as

$$\frac{G_{el}(\alpha)}{Z_{H,max}} = RT \int_{\alpha}^{1} \frac{e\psi_b}{kT} d\alpha \qquad (6.16)$$

where ψ_b is the electrostatic potential at the place on the macroion where the proton associates (e.g. *Nagasawa, 1971*).

From equation 6.16 it follows that the electrical free enthalpy of a polyelectrolyte can be obtained by charging the macroion from the uncharged state to the actual degree of ionization ($1-\alpha$). When $\psi_b(\alpha)$ can be calculated correctly, equation 6.16 and 6.13 should give the same results. This is what many publications on polyelectrolyte titrations are dealing about (e.g. *Nagasawa, 1971*).

In a polylysine helix or β structure the amino groups are closer together than in the coil conformation. Hence, ΔG_{el} of helical rods exceeds ΔG_{el} of PL coils. Therefore for polyelectrolytes exhibiting a conformational transition, a plot of pK_{app} vs α shows two distinct parts with different slopes (see fig. 6.8): At low α the curve applies to PL in the coil conformation, at high α the curve is that for the helical structure. The transition region between these parts is the coil-to helix transition region (see also section 3.1.2).

Among others *Zimm and Rice (1960)* and *Nagasawa (1971)* showed that the non-electrostatic part of the free enthalpy change of the helix-coil transition of ionic polypeptides (ΔG^{o}_{conf}) can be obtained from the curve pK_{app} vs α as:

$$\Delta G^{o}_{conf} = 2.303\ Z_{H,max}\ RT \int_{o}^{1} [pK_{app}(helix) - pK_{app}(coil)]\, d\alpha \qquad (6.17)$$

This equation is identical to equation 6.4.

As the required plots of pK_{app}(helix) and pK_{app}(coil) vs α cannot be measured over the entire α range an extrapolation procedure must be adopted to calculate ΔG^{o}_{conf}, or s.

In synthetic polyelectrolytes other than charged polyaminoacids conformational transitions are not often observed. An exception to this is the conformational transition in polymethacrylic acid (PMA) and the methacrylic acid methyl esther copolymer PMA-pe.
On charging these polymers a transition occurs from the compact, so-called hypercoiled form (a) to the common extended conformation (b). Also here ΔG^{o}_{conf} can be calculated with (6.17) (*Leyte and Mandel, 1964*).

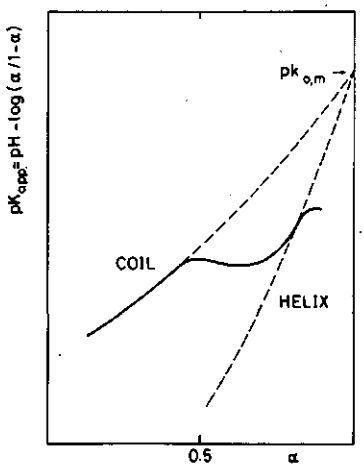


Fig. 6.8 Schematic titration curve for poly-L-lysine.

The transition in PMA-pe does also take place when the polyelectrolyte is adsorbed on paraffin droplets (*van Vliet and Lyklema, 1978*).

In section 6.6.4.1 and section 6.6.4.2 the proton titrations of PL-L and PL-DL will be compared with the corresponding solution titrations, in order to trace conformational transitions in the adsorbed state, pK shifts due to adsorption and as a consequence of adsorption, the amount of untitratable groups in a certain pH region.

Now first some other aspects of proton titration in heterogeneous media will be discussed.

6.6.2.1 Suspension effect in polyelectrolyte/charged particle systems

When measured pH values are to be used in the calculation of pK values one must be aware of the meaning of the measured pH value. In simple electrolyte solutions the meaning of the measured pH value is usually taken as $pH = -\log(H^+)$ in which (H^+) is the proton activity of the solution (e.g. *Bates, 1964*).

As the liquid junction potential is about the same in the various buffers used and the measuring solution, this definition is justified (see *Bates, 1964*). However, when highly charged particles are present a potential difference can be observed when the irreversible reference

electrode is positioned in the particle suspension or in the supernatant or dialysate of that suspension, irrespective the position of the glass electrode. The difference between the pH observed with the ref.electrode in the suspension and the real pH is called the suspension effect.

The effect is equivalent to the difference in electrical potential between the two salt bridges of two identical electrodes inserted in a suspension and its equilibrium liquid. The suspension effect is due to unequal mobilities and concentrations of cations and anions in the neighbourhood of the salt bridge tip and is therefore a liquid junction potential (*Honig, 1972; Overbeek, 1953*).

According to *Overbeek (1953)* the liquid junction potential is equivalent to the potential of the Donnan cell, i.e. the measured potential difference between the particle suspension and its equilibrium liquid thus

$$pH = pH_{obs.} - \frac{\Delta E_D . F}{2.3\ RT} \qquad (6.18)$$

where E_D, the measured potential of the Donnan cell, has the same sign as the charge of the polyion. The magnitude of the suspension effect is dependent on the charged particle concentration and small electrolyte concentration. For highly charged particles, the mobility differences between the microions are the dominant contribution to the liquid junction potential. In that case the potential of the Donnan cell can be approximately calculated from (*Overbeek, 1953*).

$$\Delta E_d = \frac{RT}{F} \ln \frac{\kappa_{susp.}}{\kappa_{solut.}} \qquad (6.19)$$

in which κ is the specific conductivity. At salt concentrations appreciably higher than the equivalent concentration of particle charges, the suspension effect is negligibly small. For this reason and also for practical ones, most proton titrations of polyelectrolytes in this study (overall polyelectrolyte concentrations usually < 0.0025 rM.) have been performed in the presence of 0.1 M NaBr.
More details concerning the suspension effect can be found in the extensive review on the subject of *Chernoberezhskii (1982)*.

6.6.2.2 *pK_{app} and the distribution of fixed charges in adsorbed polyelectrolytes*

The observed pK_{app} of a class of groups with the same $pK_{o,m}$ is dependent on the low M electrolyte concentration, the polyelectrolyte segment-segment interaction, the pH and the value of $pK_{o,m}$. Now I will pay attention to the first two effects mentioned, in relation to the proton titrations of dissolved and adsorbed PL at the same overall residue and salt concentration. Under these conditions one must realize that the distribution of fixed polymer charge (i.e. the titratable groups) will be completely different in both cases.

As the polymer volume fraction in the adsorbed layer is much higher than that in the corresponding solution, the titration curves are also expected to differ. Because of the higher electrostatic segmental interaction in the adsorbed layer pK_{app} is expected to shift to lower values in the adsorbed state. The above was also noticed by *Buscall and Corner (1982)*, who measured the titration curves of latices with a grafted PAA layer.

The concentration effect due to adsorption can be simulated by measuring the pK_{app} vs α curves of PL at various (high) residue concentrations as Buscall and Corner did for PAA. It must be realized then, that at polymer concentrations of a few % the suspension effect may be substantial even at 0.1 M salt. To obtain correct results the pH must be measured with the salt bridge of the ref. electrode in the equilibrium liquid. In the case of adsorbed polylysine and PL in solution, the ref. electrode with salt-bridge was in the suspension and not in the equilibrium liquid, but here the suspension effect is small ($c_{PL} << c_{NaBr}$), hence the measured pH is to a good approximation equal to the pH of the equilibrium liquid.

Buscall and Corner (1982) were aware of the above although they do not mention the suspension effect explicitly. They measured the pK_{app} vs α curves of PAA at 0.01 M NaCl from 0.1-5% (w/v), with the reference electrode in the polyelectrolyte solution. The curves were recalculated to obtain the pK_{app} vs α curves as if they were measured in the equilibrium liquid. This was achieved by treating the equilibrium between the PAA solution and the equilibrium solution as a Donnan equilibrium under conditions simulating the adsorbed PAA layer - solution equilibrium. The recalculated pK_{app} vs α curves were found to shift to higher pK_{app} with increasing PAA concentration as expected and not to decrease as in the untreated measurements. Although the sign of $\partial pK_{app}/\partial c_{PAA}$ in

the equilibrium liquid is predicted correctly by Buscall and Corner from the measurements in the PAA solutions, the magnitude of this quantity is probably in error and so is their estimate of the average volume fraction PAA in the adsorbed layer. The main objections are:

i. The assumption that the mean residue concentration in the adsorbed layer is much larger than the added NaCl concentration is not consistent with the estimated volume fraction in the adsorbed layer.

ii. The assumptions made about the activity coefficient of the polyion in the adsorbed layer from ion condensation theory are only valid at high dilution and not at the fairly high concentrations in the adsorbed layer.

May be the investigation of the influence of the bulk polyelectrolyte concentration on pK_{app} can be best performed using a reversible reference electrode without salt bridge. However when a saltbridge is necessarily, the measurements should be done in the equilibrium liquid.

6.2.2.3 Medium effect on $pK_{o,m}$

The $pK_{o,m}$ value of an $-NH_3^+$ group in an adsorbed PL-layer will differ from the solution value because of the following:

i. The negative PS surface charge is also present when PL is uncharged. This causes a shift to higher $pK_{o,m}$ values.

ii. The cationic groups of an adsorbed polycation in the first adsorption layer will be in an polystyrene environment and consequently experience a lower dielectric constant than in the bulk. This results in a lower value of $pK_{o,m}$.

iii. Due to the high volume fraction of PL in the adsorbed layer the mean dielectric constant is also shifted to lower values in the adsorbed layer. In fact there is a distribution of ε values. The effect on $pK_{o,m}$ is the same as that mentioned under 2, but probably less important.

When extrapolations to zero adsorbed amount and zero surface charge are made only the effect mentioned under 2 is left. Therefore strictly only that effect on $pK_{o,m}$ is a medium effect and appears also in the value of $K_{a,m}$.

When titration measurements are performed on latices with adsorbed PL at 0.1 M NaBr the suspension effect will be small as pointed out earlier.

The change in $pK_{o,m}$ upon adsorption of PL is defined as

$$\Delta_{tr}pK_o = pK_{o,s} - pK_{o,w} \qquad (6.20)$$

$\Delta_{tr}pK_o$ still depends on the surface charge, σ_o and the volume fraction PL in the adsorbed layer. The medium effect $\Delta_{tr}pK_o$ will be the largest for the polymer segments in trains at the surface. In this respect the heterogeneity of the polystyrene surface plays also a role: A PL segment in the neighbourhood of surface sulphate groups will have an enhanced $pK_{o,s}$ due to ion pair formation and PL segments embedded in a PS matrix (benzyl groups) will have a decreased $pK_{o,s}$ as said before. PL groups in tails will have $pK_{o,s}$ values not very different from those in the solution.

As the $pK_{o,s}$ value at the surface can differ considerably from that in the outer adsorption layers, difficulties arise with the definition of α in the adsorbed state. The slope of a pK_{app} vs α plot in which $\alpha = \bar{Z}_H/Z_{H,max}$ (with $\bar{Z}_H$ the average amount of protons dissociated per molecule) has therefore not the same meaning as in equation 6.10 where $\alpha = \bar{Z}_H/Z^x_{H,max}$ in which $Z^x_{H,max}$ is the total amount of groups of class x with the same $pK_{o,m}$ value.

An impression of the effect of the dielectric constant on the $pK_{o,m}$ of cationic acids can be obtained from the data of *Grunwald et al.* (see *King (1965)* p. 195). The $pK_{o,w}$ (ε = 78.3) of methylammonium is 10.70, whereas $pK_{o,m}$ in 65% methanol (ε = 40.6) is 9.58. This corresponds to a degree of dissociation α at pH 11.0 of 0.67 and 0.96 respectively. So for adsorbed PL at σ_o = o there should be a markedly higher α at pH 11.0 than in the solution. However, as will be shown later on, the surface charge σ_o is the dominant factor which determines the average shift in $pK_{o,m}$ of adsorbed PL in experimental situations. This shift results in a lower α at pH 11.0 in the case of adsorbed PL.

The theoretical description of the surface charge effect on the dissociation of ε-NH_3^+ groups can be done in different ways. When the effect is regarded as due to ion pair formation (i.e. discrete charge model) which, as was shown in chapter 5, is the case indeed at low ionic strength, the effect can be included in $pK_{o,m}$. The description will be difficult because except ε at the surface also the acidity quotients and ion pair dissociation quotients of $-OSO_3^-...Na^+$; $-OSO_3^-...NH_3^+$-R and $-NH_3^+...Br^-$ must be known, together with the appropriate statistical factors.

When the surface charge is considered as homogeneous (smeared out surface charge model) as in the polyelectrolyte adsorption theory of *van der Schee (1984)* the charge effect can be included in the electrostatic potential calculations and need not be included in the assumed value of $pK_{o,m}$.

6.6.3 Experimental

6.6.3.1 Materials

The polylysine.HBr samples used in the titration experiments have been described in section 3.1.1.

The polyglutamic acid sample used was obtained from the Sigma Co. and used without further treatment.

The PMA-pe used ($M_v 10^6$) was a sample manufactured by Röhm, A.G., Darmstadt, Germany and commercially available as Rohagit S, high viscosity grade.

Solutions of PMA-pe Na salt were prepared in the dark as described by *van Vliet (1977)*. The solution used was dialyzed against water to obtain a NaCl-free PMA-pe solution.

The concentration of PL.HBr was determined by titration with polyvinylsulphate as described before (section 4.2.2).

Adsorbed or desorbed amounts of PL.HBr were determined with the depletion method as described in section 4.2.3 and section 6.5.2. The polystyrene latex samples used in the titration experiments have been described in section 3.2. The water used was distilled once or it was conductivity water. All other chemicals used were of analytical grade.

6.6.3.2 Potentiometric titrations

Potentiometric titrations of PL, PMA-pe and PGA in solution and adsorbed on PS latex particles were performed under N_2 atmosphere in a well-closed double walled titration vessel (∿ 40 cm^3) with thermostatted water being circulated through the jacket at 293.15 ± 0.05K. Usually the titration volume was 20.0 cm^3. The N_2 used was CO_2-free and saturated with water vapour of 293 K. To that end the N_2 was passed through a soda lime column and wash bottles filled with 30% KOH, dilute H_2SO_4 and water (293.15 ± 0.05 K) respectively. The titration vessel was kept under the slight N_2 overpressure of ca. 30 mm water during the titrations. During a titration the purified N_2 was continuously passed over the solution and allowed to escape through a waterlock or wash bottle containing a solution of $Ca(OH)_2$. The solutions were stirred by means of a magnetic stirrer.

The pH was measured with an Ankersmith A161 or Electrofact 36060 digital pH meter, using combined glass-Ag/AgCl electrodes from Schott (N58 or N59) or Electrofact (7GR111). The pH meter was standardized

before each titration with Titrisol (Merck) buffers pH 7.00, 4.00 and 9.00.

Additions of calibrated 0.1 M NaOH or 0.1 M HCl (titrisol) were done with Mettler DV10/DV201 or Methrohm 655 Dosimat automatic microburettes via a rubber cap in the cover of the titration vessel. Dependent on the pH range, additions of 5-40 mm^3 0.1 M NaOH or 0.1 M HCl were made in the titration experiments. After each addition it took 2-5 min to obtain an equilibrium pH value. During the pH readings the titration sample was not stirred. One single polyelectrolyte titration i.e. in one direction, took 2-5 hours. The amount of titrant added was corrected for by subtracting a blanc titration of the solvent (see also below). Results of titrations in the presence of PS were corrected for the volume of the polystyrene present.

6.6.3.3 Sample preparation

Polylysine solutions and solutions of polyglutamic acid for titration purposes were prepared from a weighted amount of the polyaminoacid dissolved in 0.1 M NaBr in a 50 cm^3 measuring flask, to obtain an accurately known concentration of polymer, usually between 300-500 $g.m^{-3}$. PMA-pe solutions for titration experiments were prepared by dilution of a stock PMA-pe solution (see section 6.6.3.1) with the desired NaBr solution.

Before each titration, N_2 was bubbled through the solution for at least 20 min. For comparison reasons the titrations of the polyelectrolytes in aqueous solution were performed at about the same overall concentration as those in the adsorbed state. Two different methods of sample preparation were used for the titrations of adsorbed polyelectrolytes.

Method 1

This method is analogous to the one used by *Norde (1978)* for preparing titration samples of proteins adsorbed on PS latex. Prior to mixing the polystyrene latex, polyelectrolyte solution (usually PL.HBr) and NaBr solution were adjusted to the desired pH if necessary. 30 cm^3 polystyrene latex (ca. 7%) were mixed with 10 cm^3 NaBr and 10 cm^3 PL.HBr solutions of known concentrations in 50 cm^3 Sovivel flasks with leak proof screw caps (teflon sealing). The final NaBr concentration was 0.1 M unless otherwise stated.
The total concentration polyelectrolyte was chosen in such a way as

to ensure adsorption saturation. Then the flasks were rotated end over end for several hours at 298 K to allow for adsorption equilibration. After gentle centrifugation at 293 K, the polylysine concentration in the supernatant was determined in the usual way. The loose sediment was redispersed in 20 cm^3 0.1 M NaBr of the same pH as the equilibrium pH after adsorption and rotated end over end for 16 hours.
Then the dispersion was centrifuged again. The concentration PL in the supernatant was determined again and appeared always to be very low, i.e. corresponding to a desorbed amount of less than 2-4%, which is within experimental accuracy.

Again the sediment (∿ 2 cm^3) was redispersed in 20 cm^3 0.1 M NaBr solution and N_2 was slowly bubbled through the solution for one hour to remove any CO_2 absorbed during the sample handling. Then 20.0 cm^3 of the sample was pipetted in the titration cell and N_2 was passed over the solution for another 20 min to allow the pH electrode to come into equilibrium with the suspension. The starting pH was then about 7.0. After a titration the dry weight of the latex was again determined.

Blanc titrations of latex samples without PL were also performed. These titrations (corrected for the PS volume) did not differ significantly between pH 5 and 11 from those of the same volume 0.1 M NaBr solution. Therefore the latter type of titrations was usually also used to obtain blanc corrections in the titrations of adsorbed PL (see also below).

Method 2

Purified PS latex (∿6%(w/v); H^+form) titrated with NaOH to the equivalence point pH 7, was concentrated by means of destillation under vacuum at 313 K to ca. 18% (w/v). Polylysine solutions in 0.1 M NaBr (pH ∿ 6) were prepared as described before.

In a small beaker 15.0 cm^3 PL.HBr in 0.1 M NaBr and known volumes of x cm^3 NaBr solution and y cm^3 concentrated latex both at pH ∿ 6 were mixed. The concentration of the NaBr was such that the final NaBr concentration in the mixture, corrected for the solid content of the latex, was always 0.1 M. N_2 was bubbled through the mixture for one hour. Then 20.0 cm^3 of the mixture with respect to the aqueous phase (i.e. V(total) -V(PS) = 20.0 cm^3) was brought in to the titration cell. Thus the aqueous volume was kept the same for the various titrations. In this way it was possible to prepare PL/latex samples with a varying surface coverage and -equilibrium concentration PL, but with exactly the same total amount of PL. The mixture in the titration cell was stirred under N_2 atmosphere for another 20 min, then the sample was titrated.

Titrations of concentrated latex samples containing no PL.HBr were used for the blanc corrections. In some cases these blanc titrations had a higher (15% at pH 11.00) NaOH consumption than that of a corresponding volume of electrolyte solution. Fortunately this extra amount NaOH cancels when the blanc correction is performed. That the results remain unaffected by the higher NaOH consumption of some latex samples follows also from the fact that the results obtained were the same when the blanc titration was as expected. Further the results obtained with method 2 at $\Gamma = \Gamma$ (pH6) were always consistent with those obtained with method 1.

The sample preparation at a pH of adsorption of 11.00 was analogous as described under method 2 but performed completely under N_2 atmosphere. To this end the concentrated latex and NaBr solution were pipetted in the titration cell, and titrated under N_2 up to pH 11.00. In a second smaller titration vessel a PL.HBr solution was pipetted and also titrated under N_2 to pH 11.00. Then with a syringe, a known volume of the PL solution was taken from the PL solution (pH 11.00) via a rubber cap and brought into the titration vessel containing the latex also via a rubber cap, while the latex was continuously stirred. Then the PL/latex system was left under stirring for one hour to allow for adsorption equilibrium to establish and the titration with 0.1 M HCl was started. The amount of added PL was such that the concentration in the bulk was negligibly small.

6.6.4 Results and Discussion

6.6.4.1 Data treatment

In fig. 6.9.1 and 6.9.2 the primary data of a typical titration curve for PL-L dissolved in 0.1 M NaBr and adsorbed on PS (method 1) are plotted. The corresponding blanc titrations are also shown. Experimental instead of calculated blanc titrations were used in the experiments because of observed deviations of the glass electrode above pH 10.5 in some cases. A similar argument was also used explicitly by *Marini et al. (1980)* for titrations of cytochrome C.
In the case of adsorbed PL the measured blanc curve was recalculated to that corresponding to the same aqueous volume as the PL/PS latex system. In using these blanc titrations it is implicitly assumed that the free proton activity coefficient is not significantly influenced by the presence of PL. Volume corrections for dilution effects in the

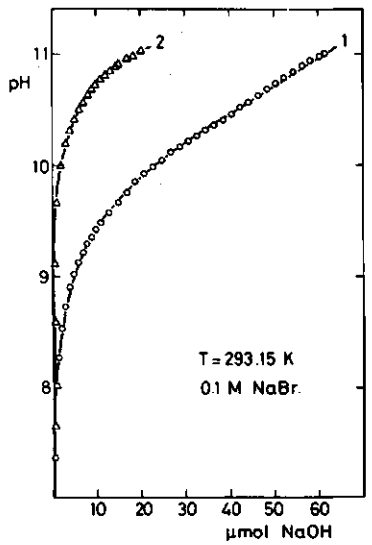


Fig. 6.9.1 1. Potentiometric proton titration of PL.HBr-L (DP 1683) c_{PL} = 586 $g.m^{-3}$ (2.82 $rmol.m^{-3}$); V_{init} = 20.0 cm^3; c_{NaBr} = 0.1 M.
2. Blanc titration of 20.0 cm^3 0.1 M NaBr.

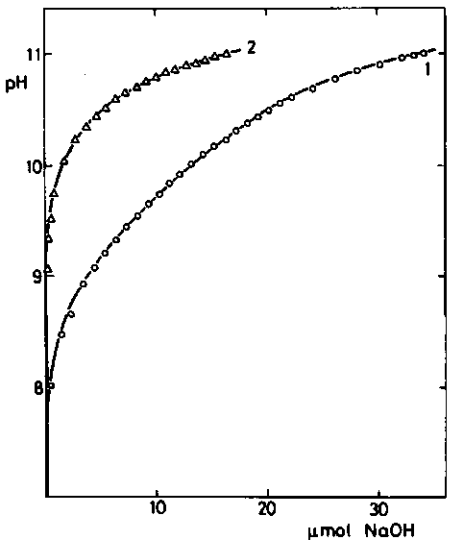


Fig. 6.9.2 1. Potentiometric proton titration of PL.HBr-L (DP 1683) adsorbed on PS (latex M_1; σ_o = -74 $mC.m^{-2}$) initial aqueous volume 18.37 cm^3; c_{NaBr} = 0.1 M. c_{PS} = 85.65 $kg.m^{-3}$; Γ_{PL} = 0.4 $mg.m^{-2}$.
2. Blanc titration of 18.37 cm^3 0.1 M NaBr.

polymer and blanc solutions due to the addition of titrant were negligibly small below pH 11.00.

It can be seen from fig. 6.9 that above pH 10.0 fairly large blanc corrections are needed. The titrations were not extended beyond pH 11.0 because the blanc corrections become then too large to obtain a reliable titration curve. In addition precipitation of PL occurs above this pH.

In principle the accuracy of the masurements can be improved when higher PL concentration can be chosen but then also higher latex concentrations are needed. At latex concentrations above 15-20%, no good stirring of the latex-PL system appeared to be possible above pH 8-9. This was due to a drastic increase in viscosity of the latex-PL system above pH 8-9. Most titration results of PL-L reported in the literature have been performed at 3-10 times higher PL-L concentration than has been used here, hence a higher accuracy can then be obtained.

In fig. 6.10.1 and 6.10.2 the titration curves of PL-L in solution and adsorbed on PS, after subtraction of the blanc titration are shown.

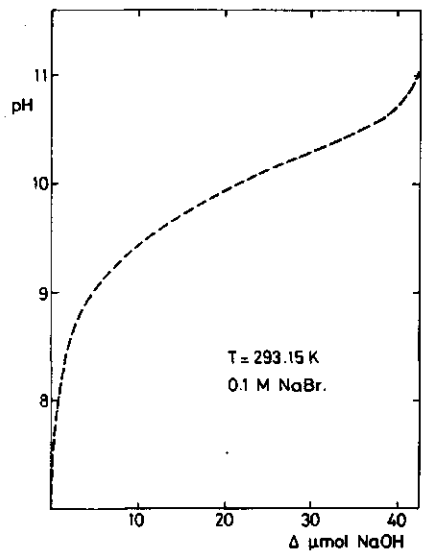


Fig. 6.10.1 Titration curve of PL.HBr-L (DP 1683) after subtraction of the amounts of NaOH used for the blanc. See further fig. 6.9.1.

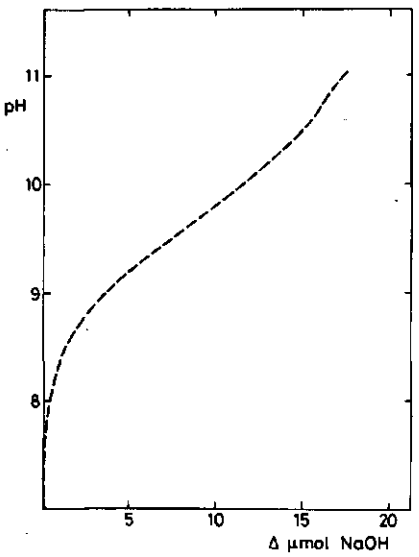


Fig. 6.10.2 Titration curve of PL.HBr-L (DP 1683) adsorbed on PS (latex M_1) after subtracting of the amount of NaOH used for the blanc. See further fig. 6.9.2.

In the PL/latex system only adsorbed PL-L is titrated because no measurable amount of PL-L is present in solution at the initial pH (see method 1).

As the maximal adsorption increases in the titration direction no desorption occurs on titration. As no significant pH changes were observed due to the PL adsorption process at low pH, there is no titration of $-NH_3^+$ groups due to adsorption. The fact that the titration curves of adsorbed PL were identical with that of the blanc between pH 3 and 7 suggest the same. Therefore the starting point of the titrations in the adsorbed state is well-defined.

As has been reported by other authors, the major experimental problem in the potentiometric titration of ionic polypeptides (or other polyacids) is the determination of the endpoint, where the polyaminoacid reaches the uncharged state. For PL this point lies above pH 12.5 and it is clearly not reached in the titrations shown here. The occurrence of precipitation and very large blanc corrections usually prevent the determination of this end point.

In order to compute α and pK_{app}, the values of titrant added where $\alpha = 0$ and $\alpha = 1$ are needed. The starting point where the titration begins ($\alpha = 0$, pH $\sim$ 7 for PL) can usually be established within narrow limits from the titration data (< 1 μmol) see fig. 6.9.

In the literature several methods for obtaining the end point (i.e. the total amount of titratable groups) are adopted by different authors. In several publications on the subject matter the method of positioning the pK_{app} vs α curves is not mentioned explicitly at all.

When ion exchanged polyelectrolytes, which are completely in the H^+ or OH^- form are used, the total amount of titrable groups can be obtained directly from the titration curve. When the titration data (pH vs Δ μmol NaOH) of a polysalt are incomplete or inaccurate in the region of the uncharged polysalt, usually a kind of iterative procedure has been adopted to obtain the total amount of titratable groups from the titration data. These methods are based on the fact that small differences in the chosen end point give markedly different pK_{app} vs α curves. One or more assumptions about the shape of the pK_{app} vs α curve (for example linearity of the helix and coil branches) are then invoked to obtain an end point satisfying the conditions chosen. See for example *Hermans (1966)* and *Tseng (1975, 1977)*. Such a procedure has also been used by *van Vliet and Lyklema (1978)* to position the titration curves of PMA-pe adsorbed on paraffin droplets.

Alternatively, α can be calculated from the titration data when the total amount of ionizable groups present is known independently, for example from the weighted amount of polymer or, in the case of polyaminoacids, from the N-content of the titration sample. The advantage is that despite the fact that no complete titration curve is available, no extra assumptions are needed to obtain pK_{app} vs α plots. However, it is implicitly assumed, that the $pK_{o,m}$ value of all titratable groups is the same in a homopolyacid, a condition which is not necessarily satisfied in the case of an adsorbed polyelectrolyte, as I will show. Then the pK_{app} vs α curves cannot be interpreted with the simple theory presented in section 6.6.2. Especially the slope of the pK_{app} vs α curves has then no clear physical meaning. In this study the following ways for the calculation of α and α' are used for the titrations of PL in solution and adsorbed:

$$\alpha' = \frac{\bar{Z}_H}{Z_{H,max}} = \frac{\Delta\mu\text{mol NaOH}}{\mu\text{mol P(res)}} \qquad (6.21)$$

in which Δμmol NaOH is obtained from plots such as given in fig. 6.10

and μmol P (res) is the total amount PL ε-amino groups present on a weight basis. The apparent pK calculated from α' and the measured pH is defined as pK'_{app} = pH-log $\frac{\alpha'}{1-\alpha'}$. Further

$$\alpha = \frac{\bar{Z}_H}{Z^{eff.}_{H.max}} \qquad (6.22)$$

in which $Z^{eff.}_{H,max} = Z_{H,max} - Z^{non}_H$, with Z^{non}_H is the amount of ionizable groups which are virtually untitratable, when compared with PL in solution in the same pH region.

For polylysine in solution it is assumed that $Z^{eff.}_{H,max} = Z_{H,max}$, so $\alpha = \alpha'$ in that case. The pK_{app} vs α curves for PMA-pe and PGA were calculated from the titration data only, in a way similar to that described by *Tseng (1975)* and *van Vliet and Lyklema (1978)*.
This was necessary because the weighted and/or adsorbed amount PMA-pe and PGA was not known accurately enough in these cases. As a consequence of this procedure approximately α is obtained and not α'.

When $pK_{o,m}$ can be taken as independent of the position in the adsorbed layer (i.e. the variation of the dielectric constant in the adsorbed layer is negligibley small) equation 6.10 can be applied to plots of pK_{app} vs α for adsorbed polyelectrolyte.

As will be shown later, the magnitude of a dip or hump in pK_{app} vs α' plots is influenced by the choice of the total amount of titratable groups, i.e. it is dependent on the definition of α.

As pointed out before, extrapolation of pK_{app} vs α curves for PL in solution to $\alpha = 1$ should give the $pK_{o,m}$ value of the ε-amino group. Of course the value found will depend on the way the extrapolation is made: linear or curved. To avoid such an ill defined extrapolation the $pK_{o,m}$ value of the PL monomer analog ε-amino-capron-amide was determined here potentiometrically. In this way extrapolations of pK_{app} vs α curves of PL towards higher α values can be made more reliable. A similar procedure was also adopted by *Terbojevich et al. (1972)* for titrations of poly-L-histidine.

The results of the ε-aminocapronamide titrations are shown in fig. 6.11.
At 293.15 K the pK_o of the ε-aminogroup is 10.79 ± 0.02 in 0.1 M NaBr and 10.63 ± 0.03 in 0.01 M NaBr.

From the data in fig. 6.10 pK_{app} was calculated and plotted as a function of α'. The curve of PL-L in solution (fig. 6.12.1) shows

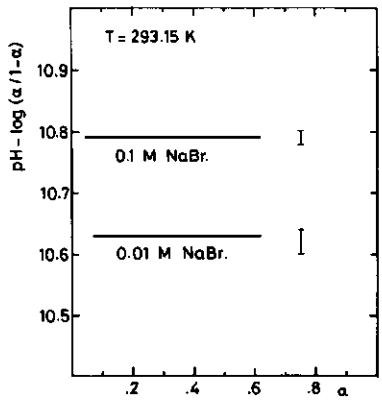


Fig. 6.11 pK vs α curves of ε-aminocapronamide, a monomer analog of PL in 0.1 and 0.01 M NaBr.

clearly the characteristic helix-coil transition. In addition it can be seen from this figure that the magnitude of the dip in the curve is strongly dependent on the actually chosen end point. In the case of adsorbed PL no transition is observed (fig. 6.12.2).

When $pK'_{app} = pH - \log \frac{\alpha'}{1-\alpha}$, is plotted against α' for adsorbed PL, the maximal value of α' is not 1.0 but lower when there is a fraction of non-titratable groups. Therefore estimations of $pK_{o,s}$ are not a priori possible by extrapolation of the pK'_{app} vs α' curves in this case.

Because of the large blanc corrections involved the pK'_{app} vs α' (or pK_{app} vs α) curves are less accurate at high α values.

The random errors in the measured pH and added μmol NaOH are small, but the pK_{app} vs α curves are subject to a systematic error, the magnitude of which differs in an uncontrolable way in repeated experiments. The consequence of this is that fairly large uncertainties are possible in the slopes of the pK_{app} vs α curves, and in the case a conformational transition occurs in the value of ΔG_{conf}, the non electrical free energy change of the transitions. Nevertheless, the trends observed in one experiment are very reproducible.

The accuracy of ΔG_{conf} determined from titration data is dependent on: 1. systematic errors in the titration procedure. 2. the choice of the end point (see fig. 6.12.1) and 3. the quality of the extrapolation procedure. Because of these factors the obtained value of ΔG_{conf} may

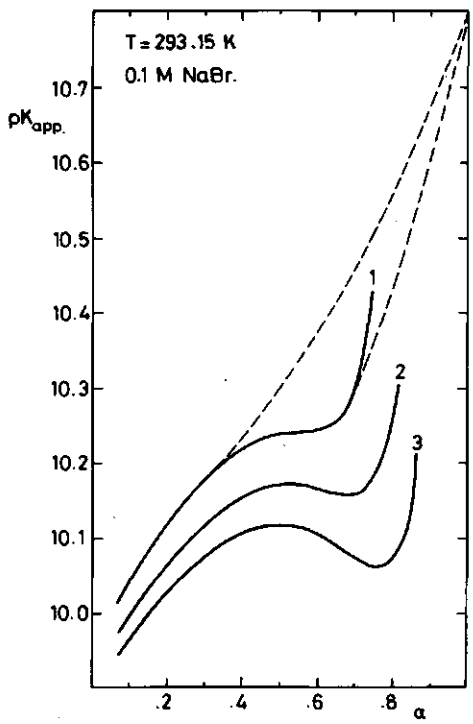


Fig. 6.12.1 pK_{app} vs α plots of PL.HBr-L (DP 1923) in 0.1 M NaBr for different values of the titration end point. c_{PL} = 2.81 $mol_r.m^{-3}$; total weighted amount of PL.HBr: 56.0 μmol_r; 1. 56.0 μmol P(res); 2. 52.0 μmol P(res); 3. 49.0 μmol P(res).

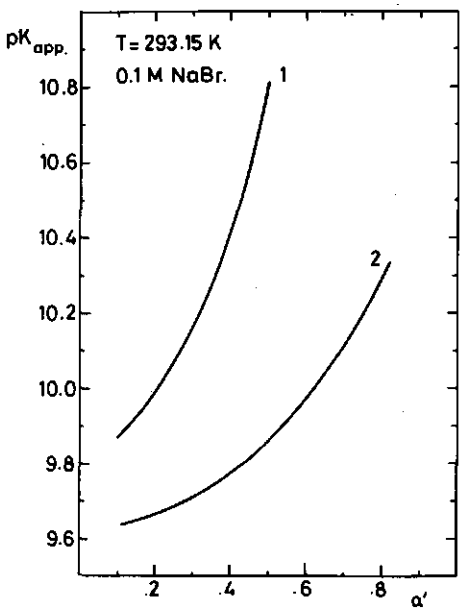


Fig. 6.12.2 pK'_{app} vs α' of PL.HBr-L (DP 1923) adsorbed on PS (latex M_1; σ_o = -70 $mC.m^{-2}$) according to method 1. c_{PS} = 85.6 $kg.m^{-3}$; Γ_{PL} = 0.4 $mg.m^{-2}$. Total adsorbed amount PL.HBr: 33 μmol_r. Curve 1: α' calculated from the total adsorbed amount and Δμmol NaOH. Curve 2: α' calculated from the total adsorbed amount, minus the total amount $-OSO_3^-$ groups present and Δμmol NaOH.

vary by a factor of 2 to 3. For further information about this matter the reader is referred to *Olander and Holtzer (1968)*, *Nagasawa and Holtzer (1964)* and to *Tseng (1975)*.

6.6.4.2 Proton titrations of free and adsorbed polylysine

Solution behaviour

In fig. 6.12.1 and fig. 6.13 pK_{app} vs α curves of PL-L and PL-DL are shown. As expected the conformational transition in PL-L is clearly visible, but PL-DL shows no transition in the investigated pH region. At about the same polymer concentration the coil part of the pK_{app} vs α plot of PL-L lies above the plot of PL-DL. This must be an effect of the different stereoregularity of the two PL forms. Such an effect of the stereoregularity has also been observed for the titration curves of isotactic and syndiotactic PAA (*Nagasawa, 1971*). However, *Chou and Scheraga (1971)* found that the coil part of the PL-L curve was identical with that for PL-DL. As expected the slopes of the PL-L and PL-DL curves in the coil region are the same within experimental error.

The data in fig. 6.13 suggest that in PL-DL the $pK_{o,w}$ of the ε-amino groups is lower than in PL-L. This is expected on the basis of data of *Ellenbogen (1952)* who found that the pK_o value of the ε-aminogroups of lysine peptides decreased by 0.1-0.4 pK units when d-lysine was incorporated in the peptide.

In one experiment the pK_{app} vs α curve of a more concentrated PL-DL solution was measured. One can see in fig. 6.13 that the pK_{app} vs α plot of this concentrated PL-DL solution lies above the curve of the more dilute one. As explained before the results must be corrected for the suspension effect at high PL-DL concentration to obtain the true concentration effect. Although the observed PL-DL concentration effect is as expected, it can not be ruled out completely that the observed difference is within experimental error. Further experiments are needed to elucidate this point further. As pointed out earlier the pH measurements ought to be performed in the equilibrium liquid.

The non-electrostatic free enthalpy of the helix formation ($\Delta G_{conf}/Z_{H,max}$) as calculated from the titration curve of PL-L in solution (fig. 6.12.1) is beteen -0.1 and -0.3 kT. The values found by other authors lie also in this range. The obtained accuracy is far too low to justify an unravelling of the various contributions to ΔG_{conf}.

As pointed out before the value of $\Delta G_{conf}/Z_{H,max}$ is small compared with the constituent contributions of among others, H-bonding and hydrophobic interactions which are partly internally compensating. In the adsorbed state, χ_s is often at least as large as $\Delta G_{conf}/Z_{H,max}$. So when a large fraction of segments is bound to a surface the occurrence of a conformational transition may become suppressed.

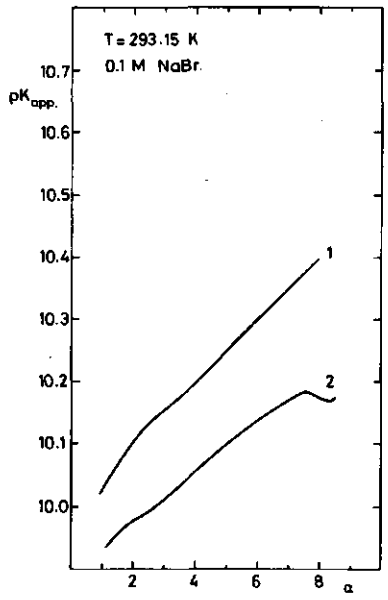


Fig. 6.13 pK_{app} vs α curves for PL-DL in 0.1 M NaBr.
1. PL-DL (DP 240) $c_{PL} = 38.4\ mol_r.m^{-3}$.
2. PL-DL (DP 308) $c_{PL} = 4.87\ mol_r.m^{-3}$.

Adsorbed poly-L-lysine

In fig. 6.12.2 pK'_{app} vs α' curves of PL-L, adsorbed according to method 1, are shown. As noted already in section 6.6.4.1 no conformational transition like the one observed in solution is present under conditions of constant adsorbed amount. As the adsorbed layer is rather flat this is not unexpected. Of course a shift of the transition to very high pH values is not excluded, but it is improbable because the fraction of trains will remain high also in the uncharged state.

Also no transition was found when PL-L was adsorbed at pH 11.0 at $\Gamma = \Gamma$ (pH 6) (method 2) first and then titrated with HCl to low pH. Unfortunately the accuracy of these experiments was not high enough to allow for conclusions about a difference in slope of the pK'_{app} vs α' curve at pH_{ads} 6 and pH_{ads} 11.0. One would expect a higher slope when at pH 11.00 PL molecules adsorb as a helix or as some other secondary structure and stay in that conformation on going to lower pH. However the 'freezing' of an adsorbed helix despite the charging process is

very unlikely, because the helix stabilization free energy is very small compared with the increasing electrostatic free energy with decreasing pH. Hence it is more likely that at pH 11.0 PL-L helices unfold upon adsorption when $\Gamma < \Gamma^{max}$ (pH 6), i.e. at very low volume fraction ϕ_*.

On the other hand one could imagine that when at pH 11.00 an adsorbed helix would be the thermodynamically most stable conformation, also at low surface coverage, this conformation is not attained during the time of measurement in the case of prior adsorption at pH 6 because of sterical constraints. The conformational properties of adsorbed PL as a function of the chain charge density and adsorbed amount will be discussed in more detail later on.

Titrations of PL-L adsorbed at 0.01 M NaBr (Γ = const) showed that also at lower salt concentration no conformational transitions occur.

In chapter 5 it was shown that the surface $-OSO_3^-$ groups at the PS surface form ion pairs with $-NH_3^+$ groups at least up to 10^{-3} M NaBr. The steep rise of curve 1 in fig. 6.12.2 shows that part of the groups belonging to $Z_{H,max}$ have a higher $pK_{o,m}$ value thus indicating that ion pair formation also occurs at 0.1 M NaBr. Further the curve indicates that the effect of the lower dielectric constant at the surface on $pK_{o,m}$ at zero surface charge is much less important than the effect of ion pair formation. As pointed out before extrapolation of curve 1 in fig. 6.12.2 to $\alpha = 1$ has no meaning.

Another indication for ion pair formation in 0.1 M NaBr stems from proton titrations that I performed on complexes of poly-L-lysine and polyvinylsulphate in 0.1 M NaBr. In this case at pH 11.00 α' appeared to be only 0.2, while for free PL $\alpha' \simeq 0.85$. When the $-OSO_3^-, -NH_3^+$ ion pairs at the PS surface with PL adsorbed are taken as effectively untitratable, α' can be recalculated as

$$\alpha = \frac{\Delta\mu\text{mol NaOH}}{\mu\text{mol P(res)} - \sigma_o . F . A_T} \qquad (6.23)$$

in which σ_o is the surface charge of the latex, F the Faraday constant and A_T the total PS surface area in the system.

Using these α values curve 2 in fig. 6.12.2 is obtained. As now the degree of dissociation is related to a class of ε-aminogroups having their $pK_{o,m}$ values probably not far apart, an analysis of the curve according to equation 6.10 is justified although not rigorous. For curve 1 this equation is certainly not applicable. Extrapolation of curve 2 (fig. 6.12.2) to $\alpha = 1$ is now possible. Such an extrapolation

results in a $pK_{o,m}$ value which is about the same as in solution, indicating that ε-aminogroups in adsorbed PL-L, not involved in ion pair formation, behave not very differently from those in solution. Unfortunately, there can be an unknown increase of σ_o due to the presence of 0.1 M NaBr as discussed in Chapter 3. For that reason the σ_o value (determined before contact with excess NaBr) used in the calculation of curve 2 (fig. 6.12.2) can be underestimated up to a factor of two. However at pH 11.00 α in curve 2 is 0.8, near to the value found in solution at that pH. Hence the underestimation of σ_o seems to be less than a factor of two.

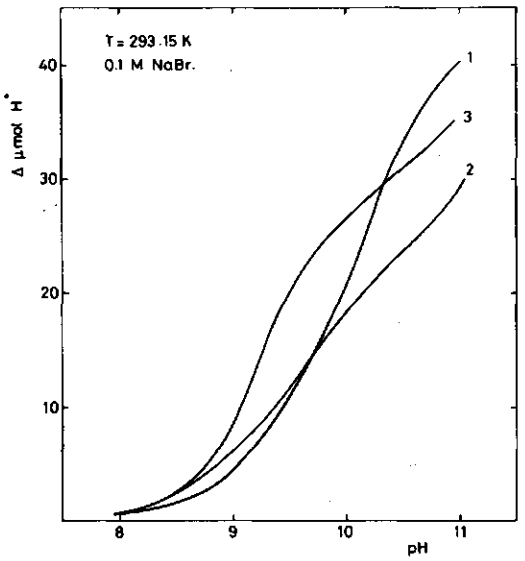
From the above it appears then that the ε-aminogroups in adsorbed PL can be divided in two classes:

1. $-NH_3^+ \ldots OSO_3^-$ groups having a $pK_{o,s}$ appreciably higher than $pK_{o,w}$ and not titrated at all under the experimental conditions.
2. $-NH_3^+$ groups having a $pK_{o,s}$ value which is about the same as $pK_{o,w}$.

The comparison of the proton titrations of adsorbed PL with the solution behaviour can be done better when sample preparation method 2 is used. In fig. 6.14.1 and 6.14.2 characteristic results of these titrations are shown. Curve 1 is the titration of PL-L (DP 1683) in solution, curve 2 the titration of PL-L adsorbed at constant adsorption ($\Gamma = \Gamma_{max}$ (pH6)) and curve 3 is the titration of adsorbed PL-L (DP 1683) with increasing adsorbed amount up to pH 9.5 and constant adsorption above that pH. The proton charge-pH curves in fig. 6.14.1 show clearly that at pH 11.00 the amount of dissociated protons decreases in the order PL-L in solution, PL adsorbed at $\Gamma = \Gamma$ (pH) and PL-L adsorbed and $\Gamma = \Gamma_{max}$ (pH 6) = constant. Hence the results obtained with sample preparation method 2 show much more clearly the fraction of aminogroups with an increased $pK_{o,m}$ value.
Curves 1 and 2 in fig. 6.14.2 show the same trends as those obtained with sample preparation method 1.

As explained before the pK'_{app} vs α' curves calculated from the data in fig. 6.14.1 may not be interpreted according to equation 6.10. This means that the slopes of these curves contain also other effects besides the consequences of the decrease in G_{el}. Furthermore, the intersection points observed in fig. 6.14.2 have no clear physical meaning.

In curve 3 of fig. 6.14.2 there is first a decrease in pK'_{app} until α' = 0.35. Above this value the curve behaves similar to curve 2, but is displaced to higher α' values. It is also above α' = 0.35 that Γ remains constant again, i.e. the bulk PL is exhausted. The question



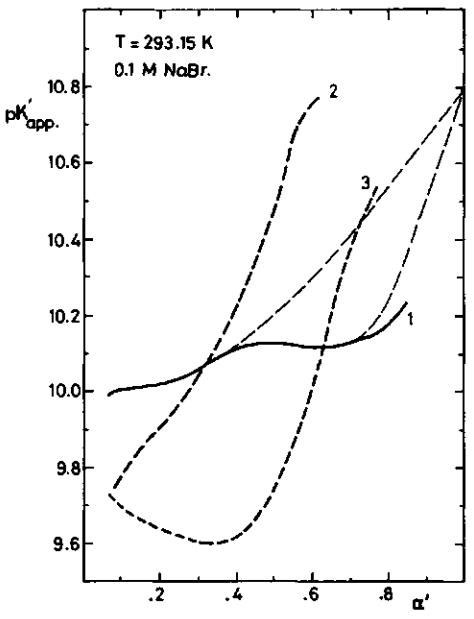


Fig. 6.14.1 Proton titrations of PL-L (DP 1683) in 0.1 M NaBr and T=293.15 K. Total amount of PL.HBr: 47.2 μmol_r (weight basis). Overall concentration PL: 2.4 $mol_r.m^{-3}$. Total amount polystyrene (latex M_5,σ_o = -39 mCm^{-2}): 1.11 g (curve 3), 2.22 g (curve 2).
1. PL-L in solution;
2. PL-L adsorbed. $\Gamma = \Gamma_{max}$ (pH 6) = const.;
3. PL-L adsorbed. $\Gamma = \Gamma$ (pH) if pH < 9.5 and $\Gamma = \Gamma$ (pH 9.5) = const. for pH > 9.5.

Fig. 6.14.2 pK'_{app} vs α' curves for the same titrations as presented in fig. 6.14.1.

now arises whether the minimum in curve 3 of fig. 6.14.2 is only caused by a coil to helix transition in the adsorbing or adsorbed PL-L molecules. To find this out we performed an titration experiment with PL-DL in solution and in the presence of PS latex under exactly the same conditions as used for the determination of curve 2 and 3

in fig. 6.14.2. The results of these titrations are shown in fig. 6.15. No minimum in the pK'_{app} vs α' plot appeared. This is evidential for the occurring of a change in the secondary structure of adsorbed PL when $\Gamma = \Gamma$ (pH) (curve 3 of fig. 6.14.2). Now it can be concluded that no coil to helix transition in the adsorbed state occurs when $\Gamma = \Gamma_{max}$ (pH 6), but that such a transition can occur when at each pH maximum adsorption is reached.

Curve 1 in fig. 6.15 represents the solution titration of PL-DL. It is comparable to the corresponding one in fig. 6.13. The curve for adsorbed PL-DL in fig. 6.15 is stronger curved than the curve for constant adsorption of PL-L in fig. 6.14.2. Also in the case of increasing adsorption of PL-DL during titration, a strong rise in pK'_{app} is observed above $\alpha' = 0.35$ just as in the corresponding case with PL-L. Also for the PL-DL/latex system the adsorbed amount stays constant above $\alpha' = 0.35$. The observed difference in behaviour between PL-L adsorbed at constant adsorption (curve 2 fig. 6.14.2) and that of PL-DL at increasing adsorption (fig. 6.15 curve 2) shows that also the adsorption process as such may be looked at as a phase transition, with its ensuing consequences for the titration curves.

In fact this is not unexpected, because the adsorption of macromolecules is a phase transition of second order, at least for infinite molecular mass (*Birshtein et al., 1979; Dimarzio and Bishop, 1974*). The sigmoidal shape of the Γ vs pH curves of PL-L and PL-DL points also in that direction.

Now I shall return to the interpretation of the pK'_{app} vs α' curves in some detail. Generally the amount of protons dissociated per polylysine molecule $\bar{Z}_H$ can be written as

$$\bar{Z}_H = \sum_{i=1}^{m} \frac{n_i K_{o,m}^{(i)} e^{y_i(\bar{Z}_H)} a_{H+}^{-1}}{1 + K_{o,m}^{(i)} e^{y_i(\bar{Z}_H)} a_{H+}^{-1}} \qquad (6.24)$$

in which there are n_i groups with a dissociation constant $K_{o,m}^{(i)}$ and potential ψ_i with respect to the bulk solution. $y_i(Z_H) = e\,\psi_i/kT$. a_{H^+} is the proton activity. From the titration results I presented above it became clear that in adsorbed PL it is a good approximation to consider just two kinds of $-NH_3^+$ groups. Those forming ion pairs with $-OSO_3^-$ groups and those who are not. Let the $pK_{o,m}$ values of these groups be

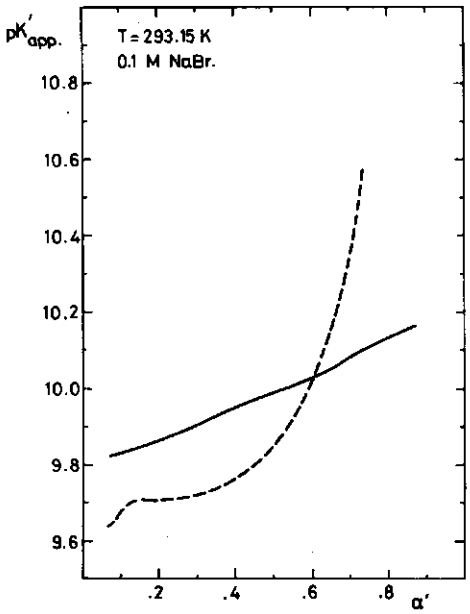


Fig. 6.15 Proton titrations of PL-DL (DP 308) in 0.1 M NaBr (curve 1) and in the presence of PS (latex M_5, σ_o = - 39 mCm^{-2}) 1.11 g, (curve 2), $\Gamma = \Gamma$ (pH), pH < 10 and Γ = const. pH > 10. Total amount of PL-DL: 48.8 μmol_r; c_{PL-DL} (overall): 2.4 $mol_r.m^{-3}$.

$pK_{o,s}^{(1)}$ and $pK_{o,s}^{(2)}$ respectively. Equation 6.24 can then be written as:

$$\bar{Z}_H = \frac{n_1 K_{o,s}^{(1)} e^{y_1(\bar{Z}_H)} a_{H+}^{-1}}{1 + K_{o,s}^{(1)} e^{y_1(\bar{Z}_H)} a_{H+}^{-1}} + \frac{n_2 K_{o,s}^{(2)} e^{y_2(\bar{Z}_H)} a_{H+}^{-1}}{1 + K_{o,s}^{(2)} e^{y_2(\bar{Z}_H)} a_{H+}^{-1}} \qquad (6.25)$$

A mean potential $\bar{y}(Z_H)$ can be substituted for $y_1(\bar{Z}_H)$ and $y_2(\bar{Z}_H)$ because the adsorbed layer is rather flat and the effect of the surface charge on $K_{o,m}$ is included in $K_{o,s}^{(1)}$. Together with

$$\alpha' = \frac{\bar{Z}_H}{Z_{H,max}} = \frac{\bar{Z}_H}{n_1+n_2}$$

and (6.26)

$$f = \frac{n_1}{n_1+n_2}$$

equation 6.25 can be written as

$$\alpha' = \frac{f\, K_{o,s}^{(1)}\, e^{\bar{y}\,(\alpha')}\, a_{H+}^{-1}}{1 + K_{o.s}^{(1)}\, e^{\bar{y}\,(\alpha')}\, a_{H+}^{-1}} + \frac{(1-f)\, K_{o,s}^{(2)}\, e^{\bar{y}\,(\alpha')}\, a_{H+}^{-1}}{1 + K_{o,s}^{(2)}\, e^{\bar{y}\,(\alpha')}\, a_{H+}^{-1}} \qquad (6.27)$$

From equation 6.27 it follows that $pK'_{app} = pH - \log \frac{\alpha'}{1-\alpha'}$, is a complicated implicit function of a_{H^+}, $pK_{o,s}^{(1)}$, $pK_{o,s}^{(2)}$, f, α' and $\bar{y}(\alpha')$. The situation would become even more complicated when a continuous distribution of $K_{o,s}$ values and y as a function of the distance from the surface has to be introduced.

From fig. 6.14.1 and table 6.1 it can be seen that in adsorbed PL-L and PL-DL at pH 11.0 there is an untitrated amount of $-NH_3^+$ groups with respect to the solution titration. The magnitude of this amount per m^2 PS surface is within the relatively large experimental error independent of Γ and it is in the same range as the surface charge of the latex. Hence the $-NH_3^+$ groups in ions pairs are virtually untitratable in the pH region 7-11 as suggested before from the data given in fig. 6.12.2. Therefore the first term in equation 6.27 can be neglected. Then the following equation applies

$$pK'_{app} = pH - \log \frac{\alpha'}{1-\alpha'} = \log \frac{1-\alpha'}{1-f-\alpha'} + pK_{o,s}^{(2)} + 0.434\, \bar{y}\,(\alpha') \qquad (6.28)$$

One now clearly sees that the strong rise of the pK'_{app} vs α' plots is caused by the first term on the right side of equation 6.28 and shows the presence of groups with strong deviating $pK_{o,m}$ within the definition of α. A similar effect can occur with the proton titrations of proteins when the amount of groups belonging to one class is taken too large (*Tanford, 1962*), something which can happen even more easily in the case of adsorbed proteins.

When $K_{o,s}^{(1)}$ and $K_{o,s}^{(2)}$ are not as far apart as in the case for adsorbed polylysine, a transition in a plot of pK'_{app} vs α' caused by the difference between $K_{o.s}^{(1)}$ and $K_{o,s}^{(2)}$ can accidently be misinterpreted as a conformational transition.

When the amount of non-titratable groups in PL adsorbed at pH 11.0 is taken equal to the amount of ion pairs then n_1, n_2 and f are known.

Table 6.1 Titration data of PL-L and PL-DL adsorbed on PS (latex M_5 σ_o = -39mCm^{-2}; A_{sp} = 9.2 m^{2}g^{-1}) and in solution. c_{NaBr} = 0.1 kmol.m^{-3}; T = 293.15 K. Sample preparation according to method 2.

	Total amount PL (μmol_r)	Total surface Area (m^2)	Titr.Am. pH 11.0 (μmol)	Non.Titr. amount pH 11.0 (mC.m^{-2})	α' pH 11.0	α pH 11.0	$(\sigma_o+\sigma_{PL})$ pH 11.0 (mC.m^{-2})
PL-L (DP1683) in solution	47.2	-	40.5	-	0.86	0.86	-
PL-DL (DP308) in solution	48.8	-	42.6	-	0.87	0.87	-
PL-L (DP1683) adsorbed at pH 6.5 on PS. Γ=const=0.48 mg.m^{-2}	47,2	20.42	29.5	+52	0.63	0.81	+43
PL-L (DP1683) adsorbed at pH 6.5 on PS. $\Gamma=\Gamma$(pH) pH<9.5 $\Gamma=\Gamma$(pH9.5)=0.96 mg.m^{-2} pH>9.5	47.2	10.21	35.5	+47	0.75	0.84	+72
PL-DL(DP308) adsorbed at pH 6.5 on PS $\Gamma=\Gamma$(pH),pH<10 $\Gamma=\Gamma$(pH10)=0.99 mg.m^{-2},pH>10	48.8	10.21	36.0	+62	0.74	0.85	+82

With $\alpha = \bar{Z}_H/n_2$ the plots in fig. 6.14.2 and 6.15 can be recalculated to obtain pK_{app} = pH - log $\alpha/1-\alpha$ vs α plots. When it is further supposed that the dielectric constant effect on $pK^{(2)}_{o,s}$ is small, the pK_{app} vs α curves obtained (fig. 6.16) are related to one class of groups i.e. with the same pK_o and hence an analysis according to equation 6.10 is warranted. A similar reasoning can be followed for, say, the car-

boxyl groups in a protein (*Tanford, 1962*). The fact that the α values at pH 11.00 are nearly the same as for PL in solution (see table 6.1) suggests indeed that $pK^{(2)}_{o,s}$ differs not much from $pK_{o,w}$.

It can be seen from fig. 6.16 that the pK_{app} vs α plots of adsorbed PL-L and PL-DL have, when Γ = const., a higher slope and lower values of pK_{app}. Both effects are due to stronger electrostatic segment-segment repulsion in the adsorbed state, compared with the bulk solution. The importance of these interactions was also found from the salt dependence of the adsorbed amount as described in Chapter 4. At higher α values the pK_{app} vs α curve of adsorbed PL crosses the corresponding solution curve. The intersection point is shifted to higher α values when Γ increases. Above the intersection point pK_{app} is higher than in solution. Only the intersection point of curve 3 and 1 in fig. 6.16 is at sufficiently low α value to be accurate enough for further interpretation. The change of sign of ΔpK_{app} (= pK_{app}(ads)-pK_{app} (bulk) at the intersection point means that the mean potential $\bar{y}(\alpha)$ changes sign with respect to the potential at the place of a dissociating group on a PL molecule in solution.

From electrokinetic measurements on PS plugs with adsorbed PL-L ($\Gamma = \Gamma_{max}$(pH 6) = const; 0.1 M NaBr) we found that the streaming potential changed sign also about pH 10.0, i.e. at about the same pH as the occurrence of the intersection point. Because at this pH the surface charge is still neutralized by $-NH_3^+$ groups (see table 6.1) the negative streaming potential found must be due to incorporation of Br^- ions. That such an incorporation of anions can occur was also inferred from the magnitude of the slopes of conductometric titrations of PS latex with PL (see chapter 5).

In principle the area enclosed between curves 3 and 4 in fig. 6.16 contains information about the non-electrostatic free enthalpy of adsorption of PL. By the same token the area between curves 4 and 5 represents the non-electrostatic free enthalpy of the conformational transition in PL near the PS surface. It can be seen from fig. 6.16 that the adsorption free enthalpy is much greater than the non-electrical free enthalpy of the helix-coil transition. Hence the intermolecular interactions between PL molecules in the adsorbed layer and the interactions of PL with the PS surface are much stronger than the intramolecular interactions responsible for the helix stability.

This suggests that the hydrophobic interaction between the alipathic parts of the PL side chains with the hydrophobic PS surface is important as was concluded before from the strong rise of the adsorbed amount

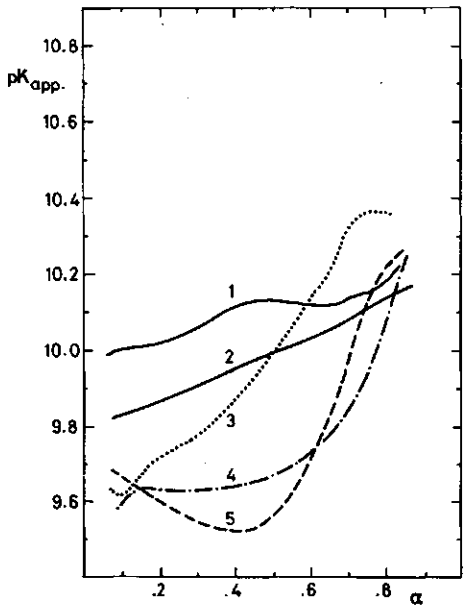


Fig. 6.16 Proton titrations of PL-L and PL-DL in solution and adsorbed on PS (latex M_5, σ_o = -39mC.m^{-2}) with $\alpha = \tilde{Z}_H / Z^{eff}_{H,max}$. 1. PL-L (DP 1683) in 0.1 M NaBr solution; 2. PL-DL (DP 308) in 0.1 M NaBr solution; 3. Adsorbed PL-L (DP 1683) $\Gamma = \Gamma_{max}$ (pH 6) = const; 4. Adsorbed PL-DL (DP 308) $\Gamma = \Gamma$ (pH) pH < 10, Γ = const (pH > 10); 5. Adsorbed PL-L (DP 1683), $\Gamma = \Gamma$ (pH) pH < 9.5, $\Gamma = \Gamma$ (pH 9.5) = const. pH > 9.5. The same titrations are plotted in fig. 6.14 and 6.15 for $\alpha' = Z_H . Z^{-1}_{H,max}$.

with increasing electrolyte concentration up to at least 1 M NaBr (see chapter 4).
In the case of a surface which can form H-bonds with the solvent or solute, such as silica, there may be a competition between intermolecular H-bonds and H-bonding with the surface, which can markedly influence the helix stability at the interface.

6.6.4.3 Comparison with other experimental systems

For sake of comparison I performed some titration experiments with PMA-pe adsorbed on positively and negatively charged PS and PGA adsorbed on positively charged latex. The sample preparation was accord-

ing to method 1, except that the pH of adsorption was about 8. The titrations were done with HCl.

In fig. 6.17 and 6.18 the pK_{app} vs α curves calculated from the data in a kind of iterative way are plotted (see section 6.6.4.1). Due to the calculation procedure the degree of dissociation obtained is about equal to $\alpha = \bar{Z}_H/Z^{eff}_{H,max}$, but the position of the curves of adsorbed PMA-pe or PGA with respect to the solution curve remains rather uncertain. Nevertheless it is striking that at intermediate α values ΔpK_{app} shows the same trends as found for adsorbed PL, at least when the signs of the charges are taken into consideration.

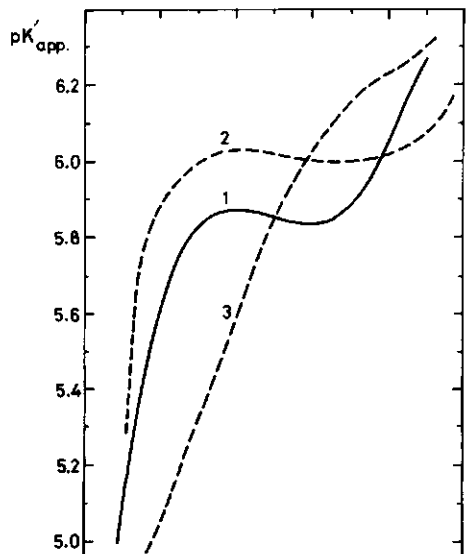


Fig. 6.17 Titration curves of PMA-pe ($M \sim 10^6$) in solution (curve 1) and adsorbed (pH ~ 8) on negatively charged PS (σ_o = -50 mC.m^{-2}) (curve 2) and positively charged latex (curve 3).
curve 1: 44.2 μmol_r PMA-pe, V = 20.0 cm^3
curve 2: 11.3 μmol_r PMA-pe, V = 19.36 cm^3, 0.825 g PS (negatively charged).
curve 3: 56.0 μmol_r PMA-pe, V = 18.47 cm^3, 1.65 g PS (positively charged).

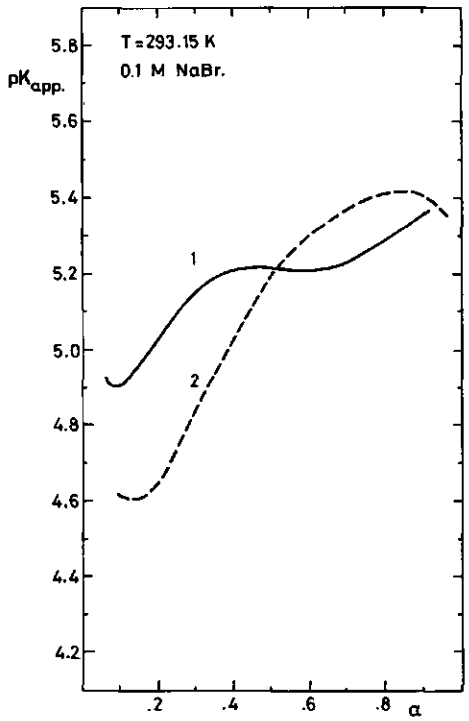


Fig. 6.18 Titrations of PGA-L (DP 150) in solution (curve 1) and adsorbed on positively charged PS latex. $\Gamma = \Gamma_{max}$ (pH 8) = const.

The only information that can be obtained with certainty from the curves is the presence or absence of a conformational transition. In solution the transition from the compact to the extended form of PMA-pe and the helix-coil transition in PGA are clearly visible. In the adsorbed state (Γ = constant) only in PMA-pe adsorbed on negatively charged PS latex a transition in the same α region as in solution is observed, while in the case of charge contrast between adsorbent and adsorbate and Γ = constant during the titration, no transition is observed. These results are in line with our observations on the PL-latex system.

In the literature only a few studies on conformational transitions in the adsorbed state are reported. *Casper et al. (1974)* found a helix-coil transition in a copolypeptide of glutamic acid and methyl gluta-

mate adsorbed at the air-water interface on changing the bulk pH, which was very similar to the transition observed in PGA solutions. *Van Vliet and Lyklema (1978)* reported a conformational transition as a function of pH in PMA-pe adsorbed on emulsion droplets. The properties of this transition were also very similar to those in aqueous solution.

Miller and Bach (1973) found that negatively charged DNA preserves its double helical structure when adsorbed on negatively charged mercury, but unfolds when adsorption takes place at positively charged mercury.

Pefferkorn et al. (1982) concluded from static and dynamic membrane properties that in PGA molecules adsorbed on a porous cellulose acetate filter a helix-coil transition still exists.

From the above it is obvious that a conformational transition at constant adsorption can take place when the surface charge and the polyelectrolyte charge have both the same sign, but when a charge contrast exist no transition at Γ = const can occur. A transition in a flat adsorbed helix is only likely to occur when the adsorption free energy per segment is of the same magnitude as the helix-stabilization free energy per segment and when there are no kinetic barriers for the transition to take place. The latter possibility is more unlikely for liquid-liquid interfaces because molecular rearrangements can occur more easily in those cases. A transition in a flat adsorbed helix will however be shifted along the pH axis with respect to the transition in solution, because the effective s value will be different in the adsorbed state. However, in the cases where a transition is observed, the characteristics are about the same as those of the transition in solution. Therefore the observed transitions in adsorbed polyelectrolytes are transitions in secondary structure of loops and tails of the adsorbed molecules. Such a transition is most likely to take place when the average loop and/or tail size is large enough to allow for any secondary structure.

In the case of polyelectrolytes the extension of the adsorbed layer depends mainly on the distance between the chargeble groups on the polymer chain and the effective adsorption energy per segment $\chi_{s,eff}$. In the cases where a transition was observed (at constant Γ) the surface charge was zero or had the same sign as the polymer charge. Probably χ_s is close to the critical value in these cases, because then the polyelectrolyte adsorption theory predicts still an appreciable adsorbed amount but the adsorbed layer is more extended than for higher χ_s values. In the case of charge contrast between adsorbate and adsorbent $\chi_{s,eff}$ will be much higher than for σ_o = 0 mC.m^{-2}. The consequence of

this is a much more flat adsorbed layer (at $\Gamma << \Gamma_{max}$) not allowing a conformational transition to occur. Also the fact that in the case of a negatively charged surface, positively charged coil segments are preferentially adsorbed plays a role in the case of charge contrast (see section 6.6.5).

The adsorbed layer of on parafin adsorbed PMA-pe is more extended than is usually found for homopolyelectrolytes, as a consequence of the emulsification process, i.e. there are long PMA-pe tails dangling in solution (*van Vliet and Lyklema, 1978*). The observed conformational transition in adsorbed PMA-pe is very likely to occur because of these tails.

When the adsorbed amount is allowed to increase with decreasing chain charge density, an adsorbed layer with longer tails and loops can be formed in which a helix-coil transition occurs as shown earlier (fig. 6.16). When PL-L is adsorbed at pH 11.0, i.e. from the helix conformation in solution, the first adsorbing molecules can be unfolded but when the adsorbed amount increases rearrangements occur, resulting in an adsorbed layer with partially helical loops and tails.

6.6.5 General discussion

The results concerning the conformational aspects of adsorbed PL-L are schematically depicted in fig. 6.19. The behaviour of adsorbed PL-L molecules is strongly determined by the presence of $-NH_3^+..OSO_3^-$ ion pairs over the whole pH region. Due to these ion pairs there is a high adsorption energy per coil segment, preventing the formation of secondary chain structure at high pH at least at low surface coverage ($\Gamma = \frac{1}{3}\Gamma_{max}$). Only when at high pH, $\Gamma \simeq \Gamma_{max}$ there may be a fraction helix in adsorbed PL-L as depicted in fig. 6.19.b.

As mentioned before the stability of a polypeptide helix as compared to that of a random coil is the result of a delicate balance of many interaction forces. At low surface coverage this balance is clearly displaced in favour of the coil.

There are several ways for reaching the adsorbed state at a chosen pH value. For example, the adsorbed state of PL-L molecules at pH 11.0 depicted in fig. 6.19.a and 6.19.b can be reached by adsorption at pH 6 first (process I) and a subsequent increase of the pH to 11.0 (process II). Alternatively the route via process III and IV can be followed. Because of the fact that the Γ-pH curves are reversible both routes will give the same adsorbed layer structure.

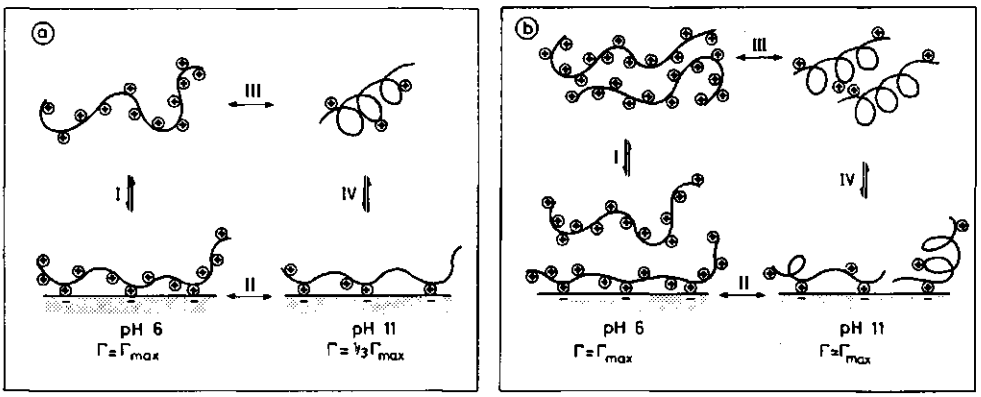


Fig. 6.19 Schematic picture of the conformational properties of adsorbed and free PL-L.

As explained earlier the results of the proton titrations at different pH of adsorption suggest strongly that also in the case shown in fig. 6.19.a the structure of the adsorbed layer is independent of the route followed.

Zhulina, Birshtein and Skvortsov (1980) investigated theoretically the adsorption behaviour of uncharged polypeptides on solid surfaces and especially the effect of secondary chain structure and helix-coil transitions on these properties. These authors considered the adsorption of infinitely long isolated chains. Hence intermolecular segment interaction is not taken into account. In most theories of helix-coil transitions in solution intermolecular interaction is also not considered. The effect of the polymer concentration is a sharpening of the transition (smaller σ) with increasing polymer volume fraction (*Grover and Zwanzig, 1974*). For adsorbed chains the neglect of intermolecular interactions is much worse than in solution in most experimental situations. This is the case because investigations concerning the secondary structure of a polyaminoacid are usually performed at rather low volume fraction in solution but necessarely high volume fraction in an adsorbed layer. However it is more relevant that the fraction of segments in trains is strongly overestimated in theories of the adsorption of single chains at the bulk volume fractions normally encoun-

tered (*Fleer and Lyklema, 1983*). In their theory *Zhulina et al.* describe a polypeptide chain in fact as a copolymer in which the chain segments can be either in a helical or a coil state.

Just as in solution the helix-coil transition is described by them with the cooperativity parameter σ and the helix growth parameter s. In the presence of an interface the effective values of σ and s are determined by the value of the adsorption energy. *Zhulina et al.* consider now three cases.

i. helix and coil segments have both the same χ_s value.
ii. a helix segment has a larger adsorption energy χ_s^h than a coil segment (χ_s^c).
iii. a helix segment has a smaller adsorption energy than a coil segment $\chi_s^h < \chi_s^c$.

In this model a completely flat adsorbed helical polypeptide has a fraction of segments at the surface of 1.0. In a real helical chain this can be $\frac{1}{3}$ or $\frac{1}{4}$ at the most. As the fraction of segments in trains is strongly overestimated in the theory of *Zhulina et al.* at the usual values for χ_s of about 1.0, they consider in fact flat adsorbed coil- and/or flat adsorbed helix sequences and the transitions between them. Hence probably only the adsorbed states depicted in fig. 6.19.a can be compared with their results, because ϕ_* is very low at pH 11.0 in that case. The situation in fig. 6.19.a is clearly a case in which $\chi_s^h < \chi_s^c$, because of the charge contrast between the adsorbate and adsorbent. In this case *Zhulina et al.* predict that adsorption leads to a strong decrease of the helical content when χ_s^c is above the critical value, which is in line with the experimental findings presented here.

In the terminology of Zhulina et al. the adsorption of PGA on the uncharged water-air interface is a case where $\chi_s^h > \chi_s^c$ because it is unfavourable to have charged coil segments adsorbed. The formation of a helix is then promoted at the surface, also in line with the existence of a transition in that case.

6.7 SUMMARY AND CONCLUSIONS

In this chapter the conformation of adsorbed polylysine was discussed together with the adsorption and precipitation properties of both poly-L-lysine and poly-DL-lysine.
It was shown that the coil to helix transition in PL-L which takes place in aqueous solution, was not present in the adsorbed state on negatively charged polystyrene latex, at low constant surface coverage.

At high surface coverage adsorbed PL-L appeared to be partly helical.

In section 6.2, the interaction forces determining the conformation of charged polyaminoacids were discussed and in section 6.3, existing theories for helix-coil transitions in charged polyaminoacids were discussed briefly. Also the possibility of secondary structure in poly-DL-aminoacids was discussed in this section.

Because at low chain charge density polylysine can precipitate from solution, the precipitation properties of PL-L and PL-DL were investigated. It appeared that the precipitation characteristics of PL-L and PL-DL were the same except for some minor differences probably connected with the absence of secondary structure in PL-DL. It was concluded from the data that PL-L shows, at least above 303 K, only one type of precipitation and not two as *Zimmerman and Mandelkern (1975 a)* found for PGA. The precipitates of PL-L and PL-DL are probably amorphous.

Also the amount of PL adsorbed as a function of the pH was the same for PL-L and PL-DL. Hence the secondary chain structure does not influence the adsorption properties. In the presence of an adsorbed layer, the precipitation of PL started at the same pH as in solution. Hence multilayer formation occurs only at worse than θ conditions.

In section 6.6 the conformation of adsorbed PL was investigated potentiometrically. After an explanation of the method, the meaning of potentiometric data of adsorbed weak polyacids was analyzed. Special attention was paid to the definition and calculation of the degree of dissociation and the implications for the analysis of the titration data. For comparison reasons also titrations of adsorbed PMA-pe and PGA were performed.

The titration results showed that, as a rule, it can be stated that at constant (low) adsorbed amount, no conformation like the one in solution occurs, when there is a charge contrast between the polyacid adsorbate and the adsorbent. This is because the effective adsorption energy per segment is high in these cases, compared with the non-electric conformational transition free energy per segment.
In the case of PL-L, ion pair formation with PS surface groups and probably also hydrophobic interactions with the surface are responsible for the unfolding of adsorbing helical molecules. Only at high adsorbed amounts, when appreciable numbers of tails and loops are present these tails and loops can be partly helical.

The titration results show further that only in the first adsorption layer the degree of dissociation α can deviate significantly from the bulk value. This deviation is largely determined by the ion pair

formation with the surface $-OSO_3^-$ groups. Outside the first layer the titration behaviour is roughly the same as it is for PL molecules in solution.

6.8 REFERENCES

Applequist, J. (1963). J. Chem. Phys. *38* (4), 934-941.

Barskaya, T.V. and Ptitsyn, O.B. (1971). Biopolymers *10*, 2181-2197.

Bates, R.G. (1964). 'Determination of pH', Wiley, New York.

Birshtein, T.M. and Ptitsyn, O.B. (1966). 'Conformations of macromolecules', Interscience Publishers, New York.

Birshtein, T.M., Zhulina, E.B. and Skvortsov, A.M. (1979). Biopolymers *18*, 1171-1186.

Buscall, R. and Corner, T. (1982). Colloids and Surfaces *5*, 333-351.

Cantor, C.R. and Schimmel, P.R. (1980). 'Biophysical chemistry part III. The behaviour of biological macromolecules', W.H. Freeman and company, San Francisco.

Casper, J., Berliner, C., Ruysschaert, J.M. and Jaffe, J. (1974). J. Colloid Interface Sci. *49*, 433-441.

Chernoberezhskii, Yu.M. (1982). 'The Suspension effect' in Surface and Colloid Science *12*, 359-453.

Chou, P.Y. and Scheraga, H.A. (1971). Biopolymers *10*, 657-680.

Ciferri, A., Puett, D., Rajagh, L. and Hermans, J. Jr. (1968). Biopolymers *6*, 1019-1036.

Conio, G., Patrone, E., Rialdi, G. and Ciferri, A. (1974). Macromolecules *7*, 654-659.

Cosani, A., Terbojevich, M., Romanin-Jacur, L. and Peggion, E. (1974) in 'Peptides polypeptides and proteins' Blout, E.R. et al. eds. J. Wiley & Sons. New York.

Davidson, B. and Fasman, G.D. (1967). Biochemistry *6*, 1616-1629.

Dimarzio, E.D. and Bishop, M. (1974). Biopolymers *13*, 2331-2348.

Doty, P. and Gratzer, W.B. (1961) in 'Polyaminoacids, polypeptides, and proteins. Stahmann, M.A. ed. University of Winconsin Press Madison.

Evers, O. (1984). MSc thesis Agricultural University Wageningen, the Netherlands.

Ellenbogen, E. (1952). J. Amer. Chem. Soc. *74*, 5198-5201.

Fasman, G.D. (1967) in 'poly-α-Amino Acids' Fasman, G.D. ed. M. Dekker Inc. New York.

Fixman, M. (1979). J. Chem. Phys. *70*, 4995-5005.

Fleer, G.J. and Lyklema, J. (1983) in 'Adsorption from solution at the solid-liquid interface'. Parfitt, G.D.; Rochester, C.H. Eds., Academic Press, London etc.

Franks, F. (1973). 'Water', Plenum Press, New York/London, *2*, Chapter 1.

Gratzer, W.B. (1967) in 'Poly-α-Aminoacids', Fasman, G.D. ed. M. Dekker Inc. New York, Chapter 5.

Grourke, M.J. and Gibbs, J.H. (1971). Biopolymers *10*, 795-808.

Grover, M.K. and Zwanzig, R. (1974). Biopolymers *13*, 2103-2115.

Hardy, P.M., Haylock, J.C., Marlborough, D.I., Rydon, H.N., Storey, H.T. and Thompson, R.C. (1971). Macromolecules *4*, 435-440.

Heitz, F. and Spach, G. (1971). Macromolecules *4*, 429-432.

Heitz, F., Lotz, B. and Spach, G. (1975). J. Mol. Biol. *92*, 1-13.

Hermans, J. Jr. (1966). J. Phys. Chem. *70*, 510-515.

Hesselink, F.Th. and Scheraga, H.A. (1972). Macromolecules *5*, 455-463.

Hesselink, F.Th., Ooi, T. and Scheraga, H.A. (1973). Macromolecules *6*, 541-552.

Honig, E.P. (1972). J. Electronal. Chem. *37*, 249-266.

Katchalsky, A. (1971). Pure Appl. Chem. *26*, 327-373.

Kauzmann, W. (1959). 'Advances in protein chemistry' *14*, 1-63.

King, E.J. (1965). 'Acid-Base equilibria' in 'The International encyclopedia of physical chemistry and chemical physics' Topic 15: equilibrium properties of electrolyte solutions vol. 4, Robinson, R.A. ed. Pergamon Press London-New York.

Klotz, I.M. and Franzen, J.S. (1962). J. Amer. Chem. Soc. *84*, 3461-3466.

Leyte, J.C. and Mandel, M. (1964). J. Polym. Sci. A *2*, 1879-1891.

Manning, G.S. (1969). J. Chem. Phys. *51*, 924-933.

Manning, G.S. (1972). Ann. Rev. Phys. Chem. *23, 117-139.*

Manning, G.S. (1978). Q. Rev. Biophys. *11*, 179-246.

Marini, M.A. and Marti, G.E. (1980). Biopolymers *19*, 885-898.

Miller, I.R. and Bach, D. (1973). Surface and Colloid Science *6*, 185-260. Matijević, E. ed.

Morawetz, H. (1965). 'Macromolecules in solution'. Interscience Publishers, New York.

Nagasawa, M. (1971). Pure Appl. Chem. *26*, 519-536.

Nagasawa, M. and Holtzer, A. (1964). J. Amer. Chem. Soc. *86*, 538-543.

Nagasawa, M. and Takahashi, A. (1972) in 'light scattering from Polymer Solutions', Huglin, M.B., ed. Academic Press, New York.

Nakagaki, M. and Ebert, G. (1982). Colloid and Polym. Sci. *260*, 781-787.

Némethy, G. and Scheraga, H.A. (1962). J. Phys. Chem. *66*, 1773-1789.

Norde, W. and Lyklema, J. (1978). J. Colloid Interface Sci. *66*, 266-276.

Norde, W. and Lyklema, J. (1984). To be published.
Olander, D.S. and Holtzer, A. (1968). J. Amer. Chem. Soc. *90*, 4549-4560.
Oosawa, F. (1957). J. Polym. Sci. *23*, 421.
Overbeek, J.Th.G. (1953). J. Colloid Sci. *8*, 593-605.
Painter, P.C. and Koenig, J.L. (1976). Biopolymers *15*, 229-240.
Pederson, D., Gabriel, D. and Hermans, J. (Jr.) (1971). Biopolymers *10*, 2133-2145.
Pfeil, W. and Privalov, P.L. (1976). Biophys. Chem. *4*, 23-32.
Poland, D. and Scheraga, H.A. (1970). 'Theory of helix-coil transitions in biopolymers'. Academic Press New York.
Prokopova, E. and Ciferri, A. (1972). Biopolymers *11*, 1621-1626.
Ptitsyn, O.B. (1971). Macromolecular microsymposia VIII & IX Praque, p. 227-244.
Puett, D., Ciferri, A., Bianchi, E. and Hermans, J. (Jr.) (1967). J. Phys. Chem. *71*, 4126-4128.
Puett, D. and Ciferri, A. (1968). Biopolymers *6*, 1213-1217.
Rosenheck, K. and Doty, P. (1961) ref. 29 of Gratzer, W.B. (1967) in 'poly-α-aminoacids', Fasman, G.D. ed. M. Dekker, Inc. New York.
Rice, S.A. and Nagasawa, M. (1961). 'polyelectrolyte solutions', Academic Press, London, New York.
Stulz, J., Müller, D., Hull, W.E. and Kricheldorf, H.R. (1983). Macromol. Chem. *184*, 1311-1322.
Schee, H. van der (1984). Doctoral thesis, Agricultural University Wageningen, the Netherlands.
Schellman, J.A. (1955). Compt. Rend. Trav. Lab. Carlsberg (Ser.chim) *29*, 223-229. Reprint (paper 7) in Poland, D. and Scheraga, H.A. (1970). 'Theory of helix-coil transitions in biopolymers' Academic Press New York.
Stigter, D. (1975). J. Colloid Interface Sci. *53*, 296-306.
Silberberg, A. (1972). J. Colloid Interface Sci. *38*, 217-226.
Tanford, C. (1962). Adv. Prot. Chem. *14*.
Tanford, C. (1973). 'The hydrophobic Effect'. J. Wiley & Sons, New York.
Tseng, Y.W. (1975). Ph.D. thesis, University of California, San Francisco.
Tseng, Y.W. and Yang, J.S. (1977). Biopolymers *16*, 921-935.
Terbojevich, M., Cosani, A., Peggion, E., Quadrifoglio, F. and Crescenzi, V. (1972). Macromolecules *5*, 622-627.
Vliet, T. van (1977). Doctoral thesis Agricultural University Wageningen, the Netherlands.
Vliet, T. van and Lyklema, J. (1978). J. Colloid Interface Sci. *63*, 97-105.

Wada, A. (1962) in 'Polyaminoacids, Polypeptides and Proteins', Stahmann, M.A., ed. Univ. Winconsin Press Madison p. 131-146.

Zimm, B.H. and Bragg, J.K. (1959). J. Chem. Phys. *31*, 526-535. Reprint (paper 17) in Poland, D. and Scheraga, H.A. (1970). 'Theory of helix-coil transitions in biopolymers' Academic Press New York.

Zimm, B.H. and Rice, S.A. (1960). Mol. Phys. *3*, 391-407.

Zimmerman, S.S. and Mandelkern, L. (1975a). Biopolymers *14*, 567-584.

Zimmerman, S.S., Clark, J.C. and Mandelkern, L. (1975b). Biopolymers *14*, 585-596.

Zhulina, E.B., Birshtein, T.M. and Skvortsov, A.M. (1980). Biopolymers *19*, 805-821.

7 POLYELECTROLYTE ADSORPTION THEORY AND THE ADSORPTION OF CHARGED POLYAMINOACIDS*

7.1 INTRODUCTION

In the previous chapters the adsorption of polylysine was considered mainly from an experimental point of view. Now the charged oligo- and polypeptides will be considered as model polyelectrolytes and some of the previously obtained experimental results will be compared with the predictions of the polyelectrolyte adsorption theory of *van der Schee (1984)*. This comparison serves several purposes:

i. Under some conditions, charged polypeptides behave as random polyelectrolytes, then they are suitable model substances to test the theoretical predictions about the influence of molecular mass, pH, and ionic strength on adsorption.

ii. Charged oligopeptide adsorption bridges the gap between adsorption of monomers and polymers, or rather between ions and polyelectrolytes. The theory for the adsorption of ions is relatively advanced and a number of equations have been put forward, such as those of Stern and Frumkin-Fowler-Guggenheim. Polymer adsorption theory is based on statistical considerations and it is useful to cover the overlap range between the two categories.

iii. Adsorption of charged polyaminoacids capable of forming secondary structures assume an intermediate position between the adsorption of flexible polyelectrolytes and proteins. It is of interest to find out the relevance of secondary structure in the theoretical description of polyaminoacid adsorption.

The substance under consideration is (mono, oligo-, poly-)lysine. The polylysines have been described in chapter 3. Oligolysines up to DP 16 were synthesized by a procedure described by *van der Schee and Tesser (1983)*. The experimental methods used and experimental results obtained have been described in detail in the previous chapters.

The adsorbed amount of PL.HBr on PS and PL.HF on AgI is expressed in mg PL.HBr per m^2 in both cases.

Special attention will be paid to the effects of pH and ionic strength (I) on the adsorption, these variables determining the charge and the screening of the charge respectively.

---

* Part of this chapter has been published in coauthorship with H.A. van der Schee and J. Lyklema. Croatica Chem. Act. 56, 695-704 (1983).

7.2 THEORY

Hitherto, no suitable polyelectrolyte adsorption theory existed. The only one available is that by *Hesselink (1977)* but it is inadequate because, among other defects, it assumes an exponential segment density-distance $\rho(z)$ relationship, thereby following Hoeve's polymer adsorption theory (*Hoeve, 1970*). Although for polymers, this is a reasonable assumption, it is incorrect for polyelectrolytes. Rather, an *ab initio* theory is needed in which ρ (z) is computed for various I and pH and the most logical approach is to extend one or more of the present day suitable polymer adsorption theories with an electrical term. Recently, this has been achieved by one of us. A more complete account is given by *van der Schee (1984)*. We shall now briefly review the principles and some important results.

As a starting point, two polymer adsorption theories may be considered, viz. those of *Roe (1974)* and *Scheutjens-Fleer (SF) (1979)*. These two theories have much in common. Both are lattice theories in which polymer configurations are formulated through step-weighted random walk statistics. The likeliness to find after j segments in a certain chain the (j + i)-th one in a given lattice layer depends essentially on three factors: (i) lattice parameters, such as the coordination number, (ii) interaction parameters with other molecules that may already be present in this layer, and in first approximation quantified through the Flory-Huggins interaction parameter χ and (iii), only for the layer adjacent to the surface (train segments) a surface interaction energy parameter χ_s. The canonical partition function Q_o is then formulated and maximalized to obtain the equilibrium distribution.

The way in which this is achieved is different in the Roe and SF theory. Roe writes Q_o in terms of $\rho(z)$ and maximizes the former with respect to the latter. In doing so, he makes an approximation that virtually amounts to the neglect of end effects (tails). The SF picture does not make this simplification, Q_o being written in terms of individual chain conformations, and also being maximized with respect to these conformations. Therefore, the Roe theories gives only $\rho(z)$, whereas SF theory distinguishes between loops and tails. This 'fine structure' has proved to be of great relevance for steric interaction because it followed from the theory that it is very likely that a number of long tails will be present in most adsorbates under conditions met in practice.

Both the Roe and SF theory have now been amplified with an electro-

static term. If it is done according to the Roe picture, the canonical partition function Q for a polyelectrolyte at a given profile $\rho(z)$ is obtained from Q_o by multiplication with exp. $(-F_{el}(\rho)/kT)$, were $F_{el}(\rho)$ is the electrical free energy of that profile $\rho(z)$. This free energy can be found, using some charging process, based on a model of the distribution of charges and their interactions. In the present case, a lattice distribution of the charges was assumed and the distribution of small ions was taken to obey Boltzmann statistics. Several charging processes were considered and their equivalence proven, more or less as double layers around particles (*Verwey and Overbeek, 1948*). If SF theory is used as the starting point, each segmental weighing factor is multiplied by an electrical term exp.$(-F\psi_i/RT)$ where ψ_i is the potential in layer i; this latter quantity being again found by some model picture.

It appears that for polyelectrolytes the difference between the ROE and SF picture is less pronounced than for uncharged polymers. The reason is that the effect of tails is less important in the charged systems. As the mathematical elaboration is more easy with the Roe picture, we shall give below only results based on this theory. A somewhat more detailed account is given by *Marra et al. (1983)*.

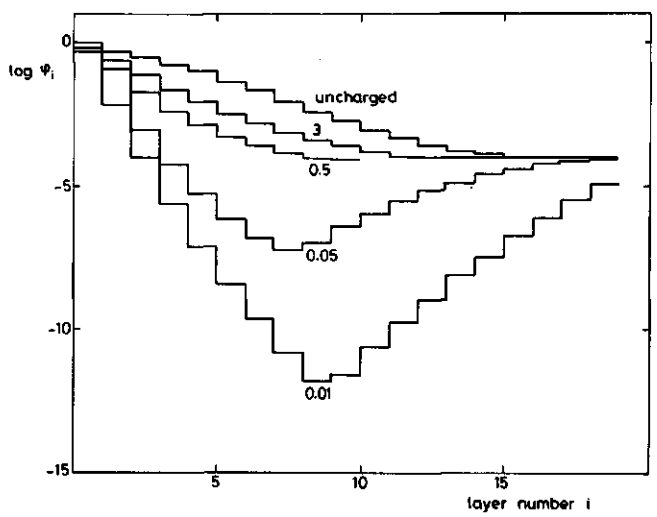


Fig. 7.1 Semilogarithmic concentration profiles for a polyelectrolyte chain of DP 240 with one charge per Bjerrumlength (0.71 nm) $\chi = 0.6$; $\chi_s = 4.85$, $\alpha_{eff} = 0.78$. Lattice site length 0.5 nm., $\sigma_o = -0.2$ charges per surface site. The ionic strength I is indicated.

Fig. 7.1 gives $\rho(z)$ for an adsorbed polyelectrolyte. As the distribution is based on a lattice picture, ρ jumps stepwise and the distance is counted in terms of layer numbers i. In fact, the ordinate gives in stead of ρ the logarithm of the volume fraction ϕ_i in each layer. The semilogarithmic plot was chosen to accentuate certain qualitative features.

In fig. 7.1 the upper curve applies to an uncharged adsorbate. The decay is almost linear, which would correspond to an exponential distribution $\rho(z)$. The deviations from linearity are a consequence of the presence of tails (for this curve SF theory was used).

Two significant new features deserve attention. The first is that at low I, a region of negative adsorption ($\phi_i < \phi_{bulk}$) occurs in the profiles. Obviously, this is due to the high repulsive potential generated by the polyelectrolyte segments present in the layers adjacent to the surface (Note that due to the logarithmic scale the depths of the minima are exaggerated). The presence of these minima demonstrates the inadequacy of polyelectrolyte adsorption theories with a pre-set $\rho(z)$.

The second feature of fig. 7.1 is that electrolytes remain effective in the range above I $\sim$ 0.1 M. Thus the theory is in agreement with the experimental observations in fig. 4.8, where the salt dependence of the PL adsorption on AgI and PS is shown. In our approach, Boltzmann statistics was employed, implying that the ions were considered point charges. Not very significantly differing results were obtained if the volumes of the ions was taken into account, using Roe's multicomponent adsorption theory (*Roe, 1974*), so that the conclusion may be drawn that the persistence of electrolyte effects up to very high concentrations is due to the fact that enough volume is available around and between the macromolecular chains to accomodate all ions.

7.3 CHOICE OF THE PARAMETERS

In this chapter we want to compare numerical results from the polyelectrolyte adsorption model of van der Schee with experimental data concerning the PL/latex, PL/AgI and PL/silica systems.

For the calculations the following parameters are needed: the lattice type, the number of segments per chain, the (non electrical) energy parameters χ and χ_s, the chain charge density and the polymer concentration. In order to convert the results to experimentally measurable quantities, the monolayer capacity must also be estimated.

7.3.1 *Coordination number of the lattice*

The model of van der Schee uses a lattice where either a segment or a solvent molecule can be placed on a site. Several lattice types are possible, e.g. a hexagonal or a cubic one.

Following *Roe (1974)* and *Scheutjens and Fleer (1979)* a hexagonal lattice was chosen. The coordination number is then twelve. Both *Roe* and *Scheutjens and Fleer* showed that the results are insensitive to this parameter.

7.3.2 *Monolayer coverage, area of a lattice site a_o and the distance between the lattice layers r_o*

The parameters used in the polyelectrolyte adsorption theory and also in the theories for uncharged polymers are essentially dimensionless. In order to compare experimental and theoretical results the system has to be scaled. One way of doing this is to calculate the parameters a_o and r_o from the specific volume of the lysyl group and its molecular mass 128.

Applequist and Doty (1961) found for the specific volume a value of 0.782 $m^3.kg^{-1}$. The volume of a lysyl residue is then 0.166 nm^3. When the lattice is isotropic, a_o is 0.3 nm^2 and r_o is 0.55 nm. This leads to a saturated monolayer coverage of 0.67 $mg.m^{-2}$ based on lysyl residues or 1 $mg.m^{-2}$ when Br^- as the counterion is incorporated.

The distance between the amino groups calculated in this way, 0.55 nm, is larger than the minimal distance between the charges when this distance is defined as *Manning (1978)* does, i.e. the distance b between the projection of the side chain groups on the linear backbone of the chain.

The value of b is then 0.36 nm (see fig. 3.1). The value obtained from the specific volume corresponds more to the actual distance between two neighbouring amino groups in the PL chain, because of the rather bulky side groups.

7.3.3 *The chain charge density*

As the distance between the charges in fully protonated PL (α=o) is smaller than the Bjerrum length in the case of a (1-1) electrolyte, counterion condensation according to *Manning (1978)* will occur (see also section 6.2.4). After condensation a net charge of ξ^{-1} remains,

i.e. one charge per Bjerrum length. The effective α is then 0.22, when 0.55 nm is taken for the distance between the amino groups. The value 0.55 nm is probably better than 0.36 nm because the PL chain is not an ideally thin rod as presupposed in the Manning theory.

In the polyelectrolyte adsorption model of van der Schee the polyelectrolyte charges are smeared out in planes parallel to the surface. This involves the assumption that charges are further away from each other than the segment length. Hence in this model the role of counterion condensation will be underestimated at least in the bulk. As a correction, effective values of α are used in the calculations, although in the adsorbed layer the premises of the Manning theory are not satisfied.

Especially the condition of an infinite, straight line charge is contradictory with the flexible polymer concept, especially so in the adsorbed layer. Further, the polyelectrolyte concentration in the adsorbed layer is not al all very dilute. Nevertheless it is assumed here that the Manning theory is approximately applicable. Above α_{eff} = 0.22 no condensation occurs and α_{eff} is identical to α again.

7.3.4 The polymer-solvent interaction parameter χ

The Flory-Huggins solvent interaction parameter χ accounts for the short range non coulombic interactions between a solvent molecule and a polymer segment.

As was shown in section 6.4 and section 6.5, PL-L and PL-DL precipitates are formed at high pH. From this effect it can be concluded that χ is greater than 0.5. For fully charged PL the theoretical adsorption isotherms are not very sensitive to the value of χ chosen (*van der Schee, 1984*). However at low chain charge density near the phase separation point this is not so (O. Evers, personal communication). For charged polylysine we chose rather arbitrarily χ = 0.6 and independent of pH, assuming that the hydration of the aminogroup is not dependent on the charge state of this group.

7.3.5 The non-electrical energy of adsorption parameter χ_s

In the case of AgI as the adsorbent the non-electrical energy of adsorption parameter χ_s was estimated by van der Schee from data obtained from coagulation experiments with the PL monomer analog and the short oligomers. To that end the χ_s parameter was adjusted in

such a way, that at the experimental determined adsorbate concentration, where coagulation takes place, the theoretical potential outside the first layer was very low. The arithmetic average of the values obtained is 4.85 (*van der Schee, 1984*). In the case of PS as the adsorbent χ_s is probably lower than for AgI, because the adsorbed amount of PL for comparable situations is lower in the case of PS (see chapter 4). Using a χ_s value of 4 it was found that a reasonable fit of the experimental results with PS was obtained.

7.4 RESULTS AND DISCUSSION

7.4.1 Dependence on electrolyte concentration

In chapters 4 and 5 it was shown that there is a strong influence of the ionic strength on the adsorption properties. This is not surprising because electrical interactions are strongly dependent on the concentration of indifferent electrolyte. Therefore the salt concentration is an important variable.

In fig. 7.2 experimental and theoretical results are shown for PL, adsorbed on AgI. The theory is capable of explaining the rise of adsorption with increasing ionic strength and it is observed that this effect continues well beyond 0.1 M.

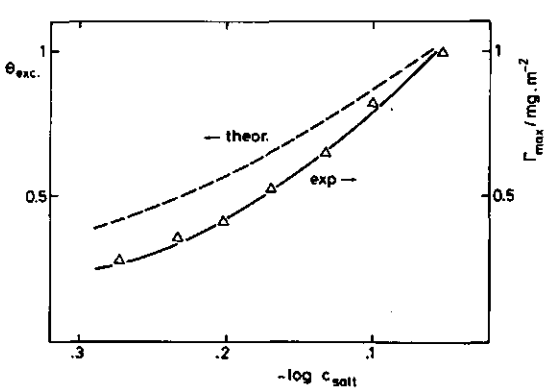


Fig. 7.2 Adsorption of poly-L-lysine on AgI. Comparison between theory and experiment. Theoretical adsorbed amounts are excesses and expressed as equivalent monolayers. Experimental conditions: pAg = 11.0; T = 293 K; pH 6; PL-L DP 300. Theoretical parameters: χ = 0.6; χ_s = 4.85; r = 300; σ_o = -0.1e/a^2, with a^2 = area per lattice site = 0.3 nm^2

Although the salt effect found for PS and AgI, which is similar, is explained well by the theory, this is not so for silica as adsorbent. In this case the Γ vs log c_{salt} plots have a maximum at constant pH and are convex at constant surface charge, this in contradistinction with the AgI on PS cases where the experimental as well as the theoretical curves are concave.

It appeared not possible to fit the results obtained with silica by adjusting χ_s. Probably the theory is not suitable to describe the salt effect in this case because χ_s is near $\chi_{s,cr}$ in this case and because ion exchange processes are relatively more important, as was shown in chapter 5. Discrete charge effects (i.e. ion pair formation) which cannot be described by the theory are important in the latter.

7.4.2 Influence of the surface charge

Theoretically it is expected that the adsorbed amount increases nearly linearly with increasing surface charge. This linearity is also found experimentally for the adsorption of PL on AgI (*van der Schee, 1984*) and also in this study for the adsorption of PL on silica. However the agreement between theory and experiment is only qualitative. Discrete charge effects may be responsible for this discrepancy.

7.4.3 Influence of the chain charge density

Although the polyelectrolyte adsorption model of *van der Schee* is not suitable for the description of the adsorption properties of weak polyelectrolytes, the surface excess as a function of the degree of dissociation can be approximately calculated when it is assumed that α does not change upon adsorption. So α is then taken as independent of the distance z to the surface. As was shown in chapter 6 this assumption is reasonable for PL segments in loops and tails.

In fig. 7.3 theoretical and experimental results are shown for PL-L DP 190 adsorbed on PS as a function of the pH. The experimental α was calculated from the total amount PL present, the added amount NaOH and the equilibrium pH. In the theory underlying fig. 7.2 it was assumed that $\alpha_{eff} = 0.22$. A consequence is that the theoretical $\vartheta_{exc.}$ is constant for values of α below 0.22. Considering the various uncertainties and approximations the correspondence between the theoretical and experimental trends is reasonable.

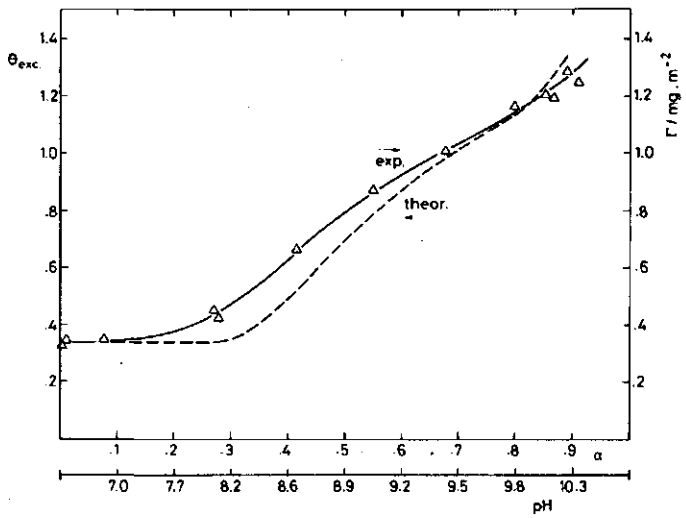


Fig. 7.3 Adsorption of poly-L-lysine-HBr on polystyrene as a function of pH (α)- Comparison between theory and experiment. Theoretical adsorbed amounts are excesses and expressed as equivalent monolayers. Experimental conditions: DP 190; σ_o (latex) = -42 mC.m^{-2}, α_{eff} = 0.22; T = 293 K; c_{NaBr} = 0.01 M. Theoretical parameters: cubic lattice; χ = 0.6; χ_s = 4; r = 300; σ_o = -0.1 e/a^2, with a^2 = 0.3 nm^2, I = 10^{-2} M.

At high α the measurements indicate that precipitation occurs (see fig. 6.2). No suitable calculations are available in the region of α = 1, so no comparison with theoretical results in the phase separation region is possible at the moment.

In fact it is remarkable that a theory for the adsorption of flexible polyelectrolytes predicts reasonably well the θ_{exc} (α) curves of a polymer which is very likely to be partially helical in the adsorbed state at high pH and Γ_{max}. Apparently the PL molecules adsorbed at high pH behave as if they are more flexible than PL molecules in the bulk, in accordance with the observation that PL is only partially helical at Γ_{max} (pH 11). This is also in agreement with the fact that the adsorption behaviour of PL-L and PL-DL is the same.

The polyelectrolyte adsorption theory can be improved when α is considered to vary with z. Also the variation of the dielectric constant with z can be taken into account (O. Evers personal communication).

The general conclusion is that the agreement between theory and experiment is sufficiently satisfactory to consider the chosen approach as a promising development, warranting further elaborations.

7.5 REFERENCES

Applequist, J. and Doty, P. (1961), in 'Polyaminoacids, Polypeptides and Proteins', Stahmann, M.A., ed. Univ. Winconsin Press. Madison.

Hesselink, F.Th. (1977). J. Colloid Interface Sci. *71*, 448-466.

Hoeve, C.A.J. (1970). J. Polym. Sci. C*30*, 361.

Manning, G.S. (1978). Quart. Rev. Biophys. *11*, 179-246.

Marra, J., Schee, H.A. van der, Fleer, G.J. and Lyklema, J. (1983) in 'Adsorption from Solution', Ottewill, A.H.; Rochester, C.H. and Smith, A.L., eds. Acad. Press. 245-258.

Roe, R.J. (1974). J. Chem. Phys. *60*, 4192-4207.

Schee, H.A. van der (1984). Doctoral thesis, Agricultural University Wageningen, The Netherlands.

Schee, H.A. van der and Tesser, G.I. (1983). Colloid Polym. J. *261*, 461.

Scheutjens, J.M.H.M. and Fleer, G.J. (1979). J. Phys. Chem. *83*, 1619-1635, *84* (1980) 178-190.

Verwey, E.J.W. and Overbeek, J.Th.G. (1948). 'Theory of the Stability of lyophobic Colloids', Elsevier, chapter III.

SUMMARY

The purpose of this study was to obtain insight in the characteristics of weakly basic (or acidic) polyaminoacids at solid-liquid interfaces. A study of polyelectrolyte properties as well as secondary structure at the interface was intended.

In these respects poly-L-lysine (PL-L) is a very suitable adsorbate because at low pH the positively charged PL-L behaves as a highly charged, more or less flexible, polyelectrolyte. At low chain charge density, PL-L assumes an α-helical or β structure dependent on temperature. Poly-DL-lysine does not show a coil-to helix transition and therefore, it is suitable as a reference for PL-L in adsorption studies.

An outline and general background of this study is given in chapter 1. Some aspects of polyelectrolyte adsorption and polyaminoacid adsorption are reviewed in chapter 2. Chapter 3 presents a selection of relevant properties of polylysine and the adsorbents used. In the following an overview of the most important results is given.

Adsorption isotherms of poly-L-lysine and poly-DL-lysine on negatively charged polystyrene are not significantly different at low pH, but also not at high pH. Also the increase of the adsorbed amount with increasing ionic strength or pH are the same for PL-L and PL-DL. Hence, there is no influence of differences in chain flexibility and secondary structure between PL-L and PL-DL on the adsorbed amount (chapters 4 and 6).

Secondary structure plays only a minor role in the precipitation characteristics of PL-L (chapter 6). Precipitation of PL-L occurs in the presence of polystyrene latex at the same pH as it does in the bulk. This indicates that multilayer formation occurs, as theoretically expected, when the solvent becomes poor (chapter 6).

Information about the secondary structure and conformational transitions in PL-L adsorbed at polystyrene is obtained from proton-titrations of adsorbed PL-L and PL-DL (chapter 6). At constant (low) adsorbed amount, no conformational transition in PL-L, comparable to the coil to helix transition in solution occurs. The charge contrast between the polyaminoacid and the adsorbent is largely responsible for the absence of such a transition in PL-L (and also in other systems).

Only at high adsorbed amounts, when appreciable numbers of tails and loops are present, formation of secondary structure in adsorbed PL is possible. Then adsorbed PL-L can be partly helical.

Analysis of the titration results further shows that only in the first adsorption layer the degree of dissociation of the PL side groups is significantly lower than it is in the bulk. This deviation is largely determined by the presence of $-NH_3^+$ - OSO_3^- ion pairs at the surface. It is because of the formation of these ion pairs and also because of hydrophobic interactions that at high pH PL-L helices unfold upon adsorption at low surface coverage (chapter 6).

Polyelectrolyte adsorption properties of PL at low pH have been investigated with hydrophilic (silica and borosilicate glass) and hydrophobic (polystyrene (PS)(latex) and silveriodide) adsorbents. The effect of chain length, ionic strength and surface charge have been considered (chapter 4). The effect of the PL chain charge density on the adsorption has been investigated with PS as the substrate in chapter 6.

At low ionic strength fully charged PL adsorbs in a rather flat conformation. In all cases the plateau value is far less than a monolayer of PL segments and no pronounced effect of the hydrophobicity of the substrate is present. Under these conditions the adsorption is independent of the chain length. An increase of the adsorbed amount is prohibited because the formation of loops and tails is prohibited as a consequence of the strong repulsion between the PL charges.

At low ionic strength the adsorption of PL on silica and polystyrene particles was also monitored conductometrically and potentiometrically (chapter 5). Phenomenologically, the binding of PL on negatively charged particles is to a certain extent analogous to the complex formation between polycations and polyanions in homogeneous solution. Plots of conductivity against polylysine concentration shows in both cases a break. In the PL - solid particle systems the amount adsorbed in the breakpoint is always lower than the maximum adsorption determined analytically, indicating that also other interaction forces than coulombic ones are important. In most cases the adsorption of PL in the breakpoint is superequivalent, which is often not the case in homogeneous polyanion-polycation complexes. The main cause for the formation of superequivalent complexes is the rigidity of the solid particles and the relative large distance between the charges on the surface. In the breakpoint every negative charge on the surface is bound to an ammonium group of PL (forming ion pairs). However, not all $-NH_3^+$ groups are bound to a negative surface charge. Titration of the adsorbed PL with polyvinylsulphate shows that the fraction free $-NH_3^+$ groups around the breakpoint is about 0.3-0.4, indicating flat adsorp-

tion. As expected, in the case of silica the charge composition in the breakpoint is dependent on the equilibrium pH and ionic strength. A relation between the occurrence of conductometric breakpoints and iso-electric adsorption seems to exist in the case of silica.

The adsorbed amount increases with increasing negative surface charge (chapter 4). In the case of silica, a linear dependence is found, as also predicted by the polyelectrolyte adsorption theory of van der Schee (chapter 7).

The adsorbed amount increases strongly with decreasing PL chain charge density. Above pH 11.0 phase separation is found to occur. As the repulsion between PL segments reduces, more loops and tails can develop and hence higher adsorption values are reached, until phase separation occurs, when the chain charge density is too low to keep the PL dissolved. Between pH 6 and 11.0 the adsorption-pH curves of PL are predicted satisfactorily by the theory of van der Schee.

In the case of the hydrophobic adsorbents polystyrene and silver iodide the adsorbed amount increases progressively with increasing salt concentration at low pH at least up to 1.0 M (chapter 4). Also the adsorption-ionic strength curves are predicted well by the polyelectrolyt adsorption theory mentioned. In these cases loop and tail formation is possible because of the screening of the PL charges by indifferent electrolyte. The coagulation concentration of PS and AgI sols in the presence of PL is strongly dependent on the polymer concentration in the bulk. When the saturation adsorption is largely reached (i.e. large excess PL (above the saturation level) left in the bulk solution) the coagulation concentration increases with increasing PL chain length, indicating the formation of longer loops and tails.

The weakening of the attraction between positively charged $-NH_3^+$ groups and negative surface groups due to addition of electrolyte does not suppress the adsorption with increasing salt concentration because the non-ionic adsorption energy is large as a consequence of hydrophobic interactions. This is not the case with silica as the adsorbent for PL, where hydrophobic interactions between PL and the surface are absent. Therefore in this case the decrease of the effective adsorption energy can suppress the increase in adsorbed amount significantly. Indeed, no increase of the adsorbed amount, at constant surface charge, is found above 0.01 M salt (chapter 4). In line with these findings proton titrations of silica in the presence of polylysine at various ionic strengths (chapter 5) show that at higher ionic strength Na^+ ions can compete with PL $-NH_3^+$ groups for surface sites.

ACKNOWLEDGEMENTS

Most of this work was carried out in the laboratory for Physical and Colloid Chemistry of the Agricultural University, Wageningen.

The author wishes to express his thanks to:

- Prof.Dr. J. Lyklema for his discussions and valuable criticism.
- Mrs. E. Rouwendal for carefully performing a large part of the adsorption measurements and some of the titration experiments.
- Mr. H. Klunder for his assistence in the proton titrations experiments of adsorbed and free PL and Mr. J.F. Bonekamp for the help with the calculations involved with the pK_{app} vs α plots.
- Ir. P-J. Bouwmeister for performing preliminary adsorption measurements and tests of the PL titration with PVS.K and Mr. W. van Leeuwen for the adsorption measurements of PL on silica, proton titrations of the PL/silica system and precipitation measurements of PL.
- Mr. H.E. van Beek, Mr. J.L.C. Verhagen and Mr. W. van Barneveld for the construction of the parts of the titration cells.
- Mr. G. Buurman for the preparation of the figures.
- Mrs. D. Neijenhuis for the typing of the manuscript.
- The departments of Biochemistry and Organic Chemistry of the Agricultural University for granting the facilities for CD and IR spectroscopy.

Finally the helpfulness, suggestions and valuable comments of the members of the Department of Physical and Colloid Chemistry was much appreciated.

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

CD	Circular dichroism
DP	Degree of polymerization
EM	Electron microscopy
i.e.p.	Iso electric point
IR	Infrared
MB	Methylene blue
NMR	Nuclear magnetic resonance
OD	Optical density
PA	Polyaminoacid
PAA	Polyacrylic acid
PGA	Polyglutamic acid
PHIS	Poly-L-histidine
PL.HBr	Polylysine hydrobromide
PL-L	Poly-L-lysine (HBr)
PL-DL	Poly-DL-lysine (HBr)
PMA-pe	Copolymer of methacrylic acid and the methyl ester of methacrylic acid
PO	Poly-L-ornithine
PS	Polystyrene particle
PSS	Polystyrene sulphate, anionic polyelectrolyte
PVS.K	Polyvinylsulphate, potassium salt
p.z.c.	point of zero charge
r.p.m.	revolutions per minute

Symbols

A_{sp}	specific surface area
A_T	total surface area
a_o	area of a lattice site
a_{H+}	activity of hydrogen ions
b	average spacing between the charges on a fully stretched polyelectrolyte chain
c	concentration
c_s	concentration indifferent electrolyte
c_{PL}	concentration polylysine.HBr
c_{PL}^T	overall concentration PL.HBr

d		diameter
$\vec{E}$		field strength
E_D		donnan potential
e		elementary charge
f		fraction ion pairs
F		Faraday constant
ΔF_{el}		electrical free energy
G		Gibbs free energy (free enthalpy)
ΔG		change in free enthalpy
	ΔG_{conf}	free enthalpy change for the increase of a helical sequence with one unit (amide residue)
	ΔG_{init}	free enthalpy change for the initiation of a helical sequence
	ΔG_m^o	standard free enthalpy of dissociation in medium m
	ΔG_{el}	electrostatic contribution to the free enthalpy change of association of a polybase
H		enthalpy
ΔH		change in enthalpy
i		layer number, index
I		ionic strength
k		Boltzmann constant
K		dissociation constant
	$K_{a,m}$	thermodynamic dissociation constant of a (cationic) acid monomer analog in medium m
	$K_{o,m}$	dissociation quotient of a (cationic) acid monomer analog in medium m at a certain salt concentration and acid concentration (i.e., the so called intrinsic K of a polyacid)
	K_{app}	apparent dissociation constant
L_T		total persistence length of a polyelectrolyte
	l_p	non electrostatic persistence length
	l_e	electrostatic persistence length
M		molecular mass
	M_n	number average molecular mass
	M_v	viscosity average molecular mass
	M_w	weight average molecular mass
N		number of charged groups on a polyelectrolyte chain
P		pressure
p		fraction of segments of adsorbed molecules attached to the surface

R		gas constant
r_o		length of a lattice site
s		equilibrium constant for the increase of a helical sequence with one unit
T		Kelvin temperature
U		uniformity coefficient
V		volume
y		dimensionless potential
Z_H		number of dissociated groups on a cationic polyacid
$Z_{H,max}$		maximum number of dissociable groups on a cationic polyacid
	$Z^x_{H,max}$	maximum number of dissociable groups of class x with the same intrinsic dissociation constant
	$Z^{eff}_{H,max}$	$= Z_{H,max} - Z^{non}_H$ with Z^{non}_H is the amount of groups which are untitratable in an adsorbed polyelectrolyte, when compared with the solution titration in the same pH region
α		degree of dissociation: $\alpha = Z_H/Z^x_{H,max}$
	α'	degree of dissociation: $\alpha' = Z_H/Z_{H,max}$
	α_{eff}	degree of dissociation taking ion condensation into account
Γ		adsorbed amount per unit area of adsorbent
	Γ_{max}	adsorbed amount at the plateau of an adsorption isotherm
	Γ_{br}	adsorbed amount at a conductometric breakpoint
	Γ_{iso}	adsorbed amount at the point of iso-electric adsorption
ε		dielectric constant
ϑ_{exc}		excess amount adsorbed expressed in equivalent mono-layers
ϑ_M		residual charge fraction after ion condensation
κ		conductivity also reciprokal Debye length
λ		wavelength
Λ		molar conductance
μ		chemical potential, dipole moment
ξ		dimensionless ion condensation parameter
ρ		ratio between the total numbers of positively charged and negatively charged groups in a polyion-charged particle complex
	ρ_{max}	this ratio at maximum adsorption
	ρ_{br}	this ratio at a conductometric breakpoint
$\rho(z)$		polyelectrolyte space charge density as a function of the distance z from the surface

σ		equilibrium constant for the initiation of a helical sequence
σ_o		surface charge density
σ_{PL}		total polylysine charge in an adsorbed layer expressed as surface charge
τ		turbidity of a sol in the presence of polylysine and salt
	τ_o	turbidity of a stable sol without PL and without electrolyte
	τ_r	relative turbidity $\tau_r = \tau/\tau_o$
ϕ		volume fraction polymer
	ϕ_i	polymer volume fraction of layer i
	ϕ_*	idem in the bulk solution
χ		Flory-Huggins solvent quality parameter
	χ_{cr}	idem, critical
χ_s		polymer adsorption energy parameter
	$\chi_{s,cr}$	critical value of χ_s
	$\chi_{s,eff}$	idem, effective
	χ_s^h	χ_s of a helical segment
	χ_s^c	χ_s of a coil segment
ψ		potential
	$\psi(x)$	potential as a function of distance
	ψ_i	potential at the lattice center of layer i, or potential at the place of ionizable groups i

DE ADSORPTIE VAN POLY-L-LYSINE EN POLY-DL-LYSINE AAN VAST-VLOEISTOF GRENSVLAKKEN

SAMENVATTING

In dit proefschrift is de adsorptie van de zwak basische polyaminozuren poly-L-lysine en poly-PL-lysine bestudeerd.

Onderzoek naar de adsorptie-eigenschappen van geladen polyaminozuren kan inzicht verschaffen over de eigenschappen van α-helices en andere secondaire structuren, die ook in eiwitten voorkomen, aan grensvlakken en de rol van ladingsinteracties hierin. Bovendien zijn deze macromoleculen geschikte modelpolyelectrolyten voor het onderzoek van de adsorptie van polyelectrolyten.

Poly-L-lysine is in dit verband een zeer interessante modelstof omdat dit polyaminozuur in oplossing bij lage pH een sterk positief geladen polyelectrolyt is en bij hoge pH (>10), afhankelijk van de ionsterkte en temperatuur, in de α-helix of β conformatie voorkomt. Poly-DL-lysine komt alleen in de kluwen conformatie voor. Daarom kan, door een vergelijking van poly-L en poly-DL-lysine, informatie verkregen worden over het effect van de stereoregulariteit en secondaire structuur in oplossing op de adsorptie.

In hoofdstuk 1 wordt het belang van de adsorptie van geladen macromoleculen, met name de eiwitten, voor tal van biologische en technologische processen aangegeven. In dit hoofdstuk wordt ook de opzet en achtergrond van het onderzoek geschetst.

Hoofdstuk 2 behandelt enkele algemene aspecten van polyelectrolyt adsorptie. Tevens bevat dit hoofdstuk een kort overzicht van de literatuur over de adsorptie van geladen polyaminozuren. De adsorptie van geladen polyaminozuren aan vast-vloeistof grensvlakken van niet biologische oorsprong blijkt nog slechts weinig onderzocht te zijn.

Een aantal relevante eigenschappen van de gebruikte materialen staat in hoofdstuk 3. Vooral het oplossings- en conformatiegedrag van poly-L-lysine en eigenschappen van de meest gebruikte adsorbentia, polystyreen latex, silica en borosilicaatglas, zoals specifiek oppervlak, oppervlakte lading en hydrofobiciteit, worden beschreven aan de hand van eigen metingen en literatuurgegevens.

Adsorptie experimenten bij maximale keten-ladingsdichtheid worden besproken in hoofdstuk 4. De polylysine moleculen zijn dan hoog geladen polyelectrolyten en hun adsorptiegedrag is dan vergelijkbaar met dat van andere flexibele polyelectrolyten.

De invloeden van ketenlengte, electrolytconcentratie en oppervlakte lading worden bestudeerd voor zowel hydrofiele (silica, borosilicaat glas) als hydrofobe (polystyreen, AgI) adsorbentia. In dit hoofdstuk wordt ook de kolloïdale stabiliteit tegen coagulatie door zout besproken voor polystyreen latex en AgI solen in aanwezigheid van PL.

Bij lage ionsterkte ($I < 0.01$ M) is de geadsorbeerde hoeveelheid altijd veel lager dan een monolaag van polylysine segmenten. De adsorptie neemt toe met toenemende wandlading van het adsorbens, maar hangt nagenoeg niet van de polylysine ketenlengte en de hydrofobiciteit van het adsorbens af. Een toename van de adsorptie wordt verhinderd, omdat de vorming van lussen en staarten onderdrukt wordt t.g.v. de sterke repulsie tussen de segmenten. Er zijn bij geen enkele zoutconcentratie waarneembare verschillen tussen de geadsorbeerde hoeveelheid van poly-L en poly-DL-lysine gevonden.

De invloed van de zoutconcentratie op de adsorptie is tweeledig: enerzijds neemt de repulsie tussen de PL segmenten af met toenemende zoutconcentratie, waardoor de lus- en staart-fractie kan toenemen en dus ook de adsorptie, anderzijds wordt ook de attractie tussen de PL segmenten en negatieve oppervlakte groepen minder als de zoutconcentratie toeneemt, waardoor de adsorptie kan afnemen. Bij negatief geladen polystyreen en AgI neemt de adsorptie sterk toe met toenemende zoutconcentratie, minstens tot 1.0 M zout. Bij het hydrofiele silica is, bij constante wandlading, boven 0.01 M zout, geen toename in adsorptie meer waar te nemen. Dit komt omdat bij silica geen hydrofobe attractie tussen PL segmenten en het oppervlak aanwezig is zodat de effectieve adsorptie energie per segment veel lager is dan bij PS en AgI.

De zoutconcentratie waarbij coagulatie van latex- of AgI solen optreedt in aanwezigheid van polylysine is sterk afhankelijk van de polymeerconcentratie in de bulk. De resultaten wijzen erop dat, indien de verzadigingsadsorptie ruimschoots bereikt is, de lus- en staartfractie toeneemt met de polylysine ketenlengte en hoger is dan bij lage ionsterkte.

In hoofdstuk 5 wordt de binding (i.e. adsorptie) van positief geladen polylysine aan negatief geladen kolloïdale deeltjes (polystyreen en silica) bestudeerd m.b.v. conductometrie en potentiometrie. De adsorptie van PL wordt vergeleken met de complex-vorming tussen lineaire polyanionen en lineaire polykationen. Ook worden proton-titraties van silica in aan- en afwezigheid van PL besproken. Uit deze proton titraties blijkt dat de silanol groepen sterker zuur zijn in aanwezigheid

van PL. Bij hogere zoutconcentratie kunnen Na^+ ionen, $-NH_3^+$ groepen verdringen van $-Si-O^-$ groepen aan het silica oppervlak. Dit is in overeenstemming met het feit dat de adsorptie niet meer toeneemt boven 0.01 M zout.

Evenals in het geval van opgeloste polyanionen en polykationen vertoont de geleidbaarheid als functie van de polylysine concentratie een knik. De pH is na het knikpunt constant. De geadsorbeerde hoeveelheid in het knikpunt is lager dan de maximale adsorptie. De samenstelling van het 'PL-kolloïde' complex in het knikpunt is niet ladingsstoichiometrisch en in de meeste gevallen is de PL adsorptie superequivalent. Dit komt doordat de vaste deeltjes niet flexibel zijn en de negatieve oppervlaktegroepen relatief ver van elkaar zitten. In het knikpunt is elke negatieve oppervlaktegroep gebonden aan een $-NH_3^+$ groep van PL (ion-paren), maar het omgekeerde geldt niet.

Uit titraties van aan polystyreen deeltjes geadsorbeerd PL met polyvinylsulfaat, blijkt dat de fractie vrije $-NH_3^+$ groepen 0.3-0.4 is.

Hoofdstuk 6 handelt over de secondaire structuur van geadsorbeerd poly-L-lysine en de invloed van de secondaire structuur op de adsorptie en precipitatie eigenschappen van PL. Ook wordt de invloed van de pH (i.e. dissociatie graad) op de adsorptie van PL aan polystyreen besproken.

De precipitatie-tijd en precipitatie pH van poly-L-lysine zijn bij 303 en 313K nagenoeg gelijk aan die van poly-DL-lysine. Blijkbaar heeft de secondaire structuur geen grote invloed op deze eigenschappen.

De adsorptie van PL neemt sterk toe met toenemende pH, omdat t.g.v. de afnemende repulsie tussen de segmenten de lus- en staartfractie toeneemt. Bij de pH waar ook in oplossing precipitatie optreedt, gebeurt dit ook in aanwezigheid van polystyreen latex.

Protontitraties van geadsorbeerd PL laten zien dat in het geval van constante (lage) geadsorbeerde hoeveelheid geen conformatieovergang plaatsvindt. Alleen wanneer bij elke pH de verzadigingsadsorptie bereikt is, wordt een conformatieovergang in geadsorbeerd PL waargenomen, omdat dan de lussen en staarten lang genoeg zijn voor de vorming van secondaire structuur. Bij hoge pH en maximale adsorptie is geadsorbeerd PL-L waarschijnlijk gedeeltelijk in de α-helix conformatie. T.g.v. ionpaar vorming met de negatieve oppervlakte groepen, blijkt de dissociatiegraad van geadsorbeerd polylysine alleen in de eerste adsorptielaag (treinfractie) behoorlijk lager te zijn dan in oplossing. De ontvouwing van PL-L in de α-helix conformatie t.g.v. adsorptie bij lage oppervlakte bezetting is waarschijnlijk het gevolg van de vorming van deze ion-paren en hydrofobe interacties met het polystyreen oppervlak.

In hoofdstuk 7 wordt de pH en zoutafhankelijkheid van de adsorptie van PL aan polystyreen en AgI vergeleken met de voorspellingen van de polyelectrolytadsorptietheorie van van der Schee. In deze gevallen is er een goede overeenstemming tussen experiment en theorie.

CURRICULUM VITAE

Ben Bonekamp is geboren op 25 april 1953 te Sittard. Aan het Bisschoppelijk College in die plaats behaalde hij in 1971 het diploma H.B.S.-B. In datzelfde jaar begon hij aan de Landbouwhogeschool te Wageningen de studie Moleculaire Wetenschappen.
Hierin werd in januari 1976 het kandidaatsexamen afgelegd en in september 1978 het doctoraalexamen. De doctoraalstudie omvatte de vakken: Biochemie (6 mnd), Kolloïdchemie (6 mnd), Virologie (6 mnd) en Wiskunde (3 mnd). Vanaf februari 1979 tot februari 1983 was hij in tijdelijke dienst van de Landbouwhogeschool te Wageningen. In die periode verrichtte hij, als wetenschappelijk medewerker bij de vakgroep Fysische en Kolloïdchemie met een gedeelde onderwijs- en onderzoektaak, het in dit proefschrift beschreven onderzoek.
Vanaf oktober 1983 is hij voor een periode van 1 jaar werkzaam bij de vakgroep Levensmiddelentechnologie, Sectie Zuivel en Levensmiddelennatuurkunde van de Landbouwhogeschool.

NAWOORD

Aan het eind van dit proefschrift wil ik graag iedereen bedanken die, direct of indirect, aan de totstandkoming ervan heeft bijgedragen. Een aantal mensen wil ik persoonlijk noemen:

Mijn promotor, Hans Lyklema, ben ik erkentelijk voor de nuttige discussies over de resultaten van het onderzoek en het leveren van opbouwende kritiek op de tekst van de verschillende hoofdstukken.

Erna Rouwendal en Harco Klunder wil ik bedanken voor de nauwgezette wijze waarop zij een groot aantal van de experimenten hebben uitgevoerd en mijn broer Hans, voor het verrichten van een aantal berekeningen m.b.v. zijn 'micro'.

Mijn dank gaat verder uit naar Wim van Leeuwen en Pieter-Jan Bouwmeister, die in het kader van hun doctoraalstudie hebben bijgedragen aan het experimentele werk. Tevens ben ik Hennie van Beek en Louis Verhagen erkentelijk voor de technische assistentie.

Ik dank Gert Buurman voor het tekenen van de vele figuren en het maken van de foto's en Dory Neijenhuis voor het typen van het manuscript.

Tenslotte wil ik mijn kamergenoot Thonie van den Boomgaard bedanken voor de prettige sfeer en de nuttige gesprekken. Ook de waardevolle discussies met andere medewerkers van de vakgroep Fysische en Kolloïdchemie, met name Rob Cleven, waardeer ik ten zeerste.

Op een geheel andere wijze hebben mensen uit mijn naaste omgeving, in het bijzonder Margo, de totstandkoming van dit proefschrift mede mogelijk gemaakt.